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SYMPOSIUM ON SCIENCE IN SOCIETY

BY INVITATION OF THE COMMITTEE ON ARRANGEMENTS FOR THE ANNUAL MEETING

Chairman. H. L. SHAPIRO

Read before the Academy, April 28, 1958

CONTENTS

SCIENCE IN SOCIETY . . . . . *H. L. Shapiro* 661
SOME REFLECTIONS ON THE STATE OF SCIENCE IN AMERICA DURING THE NINETEENTH CENTURY . . . . . *I. Bernard Cohen* 666
SCIENCE IN THE FRENCH REVOLUTION . . . . . *Charles C. Gillispie* 677
SOCIETY, SCIENCE, AND EDUCATION IN THE SOVIET UNION . . . . . *Chauncy D. Harris* 684

SCIENCE IN SOCIETY

By HARRY L. SHAPIRO

AMERICAN MUSEUM OF NATURAL HISTORY

This symposium, which we have boldly entitled "Science in Society," requires a word of explanation by way of preface. Although acute and prescient writers have from time to time pointed out the impact of science on society and the world we live in, it is only during the past decade or two that the consciousness of this had finally emerged into the surface of our collective thinking. Only recently have the implications begun to affect deeply the conceptualizations of human organization, of human evolution, and of the future of man. If our understanding of the dynamic influence of science on society is only in its initial stage and if our control of the situation remains elementary, the reality has become apparent to almost everyone. Both the man on the street and the scholar have been made acutely aware of the role that science now plays, not only in our national defense but in almost every department of our lives.

What still remains less generally apparent is the reciprocal relation of society on science. For if we are to understand the one, we must acknowledge the other. Neither science nor scientists function in a social vacuum whatever the popular stereotypes may suggest or the absorbed scientist may overlook. Science and scien-

tists can exist only in a social milieu and they are, therefore, inevitably affected by historical, social, and cultural events and patterns. Science as an organized activity in a complex society must adjust itself to that society. And the agents of science—the scientists—are members of a society that exercises both obvious and subtle controls on them as upon other members of society. In a very real sense the current concern with American education illustrates how social values may impinge on science itself.

This symposium might, therefore, have been more accurately called "Society in Science," but such a title without a lengthy explanatory subtitle might have evoked irrelevant overtones. The influence of society and culture on the development and course of science is necessarily complex and goes back a long way in time. We can in a single symposium, therefore, scarcely hope, in a temporal sense, to cover it at all adequately. Our aim, on the contrary, will be to explore the process in an episodic way, having selected various historic periods that provide some degree of contrast.

From another point of view we will be obliged to be equally cursory since the field we are entering is not well explored. There is in existence no established corpus of tested fact, principle, or concept. This is an experiment by invitation. Our guests, who do not profess to be experts on this particular subject, have been asked to present their views on it as they see it from their specialized fields of scholarship or out of the fullness of their experience. They were not only coöperative guests in accepting such an invitation, they were also courageous in venturing on these uncharted seas. But above all they are deserving of our gratitude since they were asked to undertake these difficult assignments on extremely short notice. Their resources have my highest admiration, since they might well have refused to embark on this venture without long preparation and reflection.

My own contribution to this joint effort will be as an anthropologist. As such I shall merely venture to offer some general background and points of view on a process that we see in our field of primitive culture only in embryo and never in the full-blown state characteristic of historic civilizations or, if we limit science rigorously, only within modern times.

In a strict sense, science as a consciously organized body of knowledge, susceptible of rational proof and demonstration and testable by experiment, did not exist in the earlier and more primitive stages of human development and does not now exist among contemporary primitive people. Having said this, I must qualify it further. So-called primitive men do possess a body of knowledge or, better, belief, which in certain respects is comparable to the place of science in our world. It differs from science in that it gives validity to phenomena demonstrable by non-rational criteria and intertwines the supernatural with the natural. Moreover, it is never organized as a conscious quest for the expansion of knowledge. It grows and changes, but not through deliberate design and organized purpose. In addition, or even as part of it, there exists in all cultures or societies a core of technological knowledge based on accumulated experience and tradition and on observation. Even at the most primitive level this technological information contains the germ of a science, since within its limits it is precise, predictable, susceptible of experimentation, and transmissible. If it were not, primitive society could not have existed and human culture could not have evolved. To take a simple example, Paleolithic man even at an early stage had to know a fair amount about the nature of flint and its cleavage patterns in

order to convert an irregular nodule by flaking technique into a useful tool that could extend significantly the efficient use of the bare hand and arm. At a later stage of development our predecessors had to acquire a modest but essential degree of reliable information about the properties of copper and tin in order to carry out the metallurgical processes that convert these ores into usable forms and that permit their combination into bronze alloys.

The superficial and inadequate knowledge that lay behind such technological skills should not blind us to the fact that a science of a sort is involved even in these relatively crude techniques. Science, therefore, or technology if you prefer, is virtually coterminous with man for it is becoming increasingly apparent—if only as a working hypothesis—that the evolution of man from a species of primate was possible only because of the acquisition of culture. And culture implies a technology.

The fossil record as new documents are added makes it clear that man's ancestors acquired the ability to maintain a habitually upright posture, consequently freeing the hand for purposes other than locomotion, before any considerable expansion of the brain had occurred. In assuming such a novel gait, the precursors of man in a sense were entering a new environmental niche. If they had survived in this hazardous situation and adapted themselves to it without further ado, following the pattern of organic evolution in the past, they would undoubtedly have acquired a number of specialized anatomical, and perhaps physiological, features to fit such an environment. Indeed, there is evidence to suggest that some of these experimenting primates followed such a line that finally led them to extinction. The distinction of human evolution and what makes it unique is the interposition of a cultural environment between emergent man and the physical world. Culture, which represented early man's extra or non-organic method of adaptation, thus relieved his body of the necessity of specialized organic adaptations. And this cultural device, proving far more responsive to rapid change than organic evolution, not only established its worth from an adaptive point of view but, in turn, became a kind of selective agent favoring those most successful in using and manipulating culture. By such a process, or by one something like it, the rapidity of human evolution becomes understandable. And even more significantly the enormous expansion of the human brain as a culturally adaptive phenomenon becomes clear.

If culture, therefore, plays so vital a role in human evolution, it is legitimate to assign to science and/or technology an indispensable and dynamic part in it. The point I am trying to make here is that science and technology from the outset of human evolution have directly and inevitably affected human evolution and as far as we can see will continue to do so.

It becomes, therefore, a reasonable query to ask what precipitated the appearance of technology in the first place and what factors have continued to affect it and through it mankind itself? It is often easier to ask such questions than to answer them. This one is no exception. We are unfortunately in no position to offer a completely adequate explanation for the emergence of technology. The psychological characteristics that encouraged the precursors of man to employ their hands in making and using tools and, in turn, handing on the traditions established to succeeding generations are not thoroughly understood and lie, in any event, beyond my competence. To an anthropologist, however, technology, and even culture in the wider sense, has meaning as a response to adaptive necessities. It is sig-

nificant that the earliest known artifacts, and for a very long time in history the only artifacts, are tools or implements useful in overcoming prey and in gaining a livelihood. Tools, therefore, may be regarded as extensions of the human body that made it possible and certainly easier for primitive man to survive. And since man at this stage was neither fleet enough of foot nor powerful enough in tooth or limb to challenge unaided the perils that his relatively conspicuous size on the ground exposed him to, he must have found extra organic weapons and tools too valuable to dispense with once employed. If I may generalize these observations, I think it would be fair to say that technology, and science in its broadest sense, arise out of and survive because of the adaptive needs of our earliest precursors struggling to maintain themselves in a new environmental niche—upright posture on the ground.

The earliest stone tools recognizable as of human manufacture are uniformly crude and relatively undifferentiated. In the course of the several hundreds of thousands of years that constitute the Paleolithic, these stone prototypes underwent both an increasing specialization of type, reflecting a wider range of tool activity, and an increasing skill in their manufacture. The two developments are obviously inter-related. Greater knowledge of the material and control of the necessary skills made possible more specialized types. And the need for more finely adapted types of instruments must have stimulated increased exercise of such skills. The progress revealed in these mute but eloquent survivals of ancient triumphs are comparable to the accumulative advance of modern science. And they reflect the demands and values of a way of life whose preoccupations were centered on such adjuncts for its success.

Although it would be debatable that the artificial cultivation of wild grain grasses was a discovery directly stimulated by an evolving culture, the archaeological evidence certainly suggests that once agriculture was adopted its rapid advance and diffusion throughout much of the world may be attributed to the demands of society. As an economic base for social organization its superiority over more primitive hunting and gathering economies was sufficiently convincing to encourage neolithic man to go to great lengths to overcome barriers to its adoption and to advance its increased productivity. To fell trees and clear forested areas, to prepare the soil and reap the harvest, and to deal with the new and varied demands of agriculture brought into existence a notable improvement and extension of tools and technological skill. Perhaps most striking are the remains of vast irrigation and water control engineering projects. Since agriculture necessarily means a kind of intensive concern with specific plots of land, a new social need for identifying, measuring, and surveying arose. This in turn led to the development of mathematics. By no means of negligible significance in the progress of this science was the influence of trade and commerce which in the neolithic began to assume considerable proportions as a result of the economic revolution following on the development of agriculture. Settled life, village organization, the production of agricultural surpluses and the development of specialized crafts provided a matrix out of which trade could develop. And commercial transactions, in turn, required a system of accounting.

Astronomy, one would think, might be as remote from the practical needs of primitive man and early civilization as any branch of science. And yet in widely

separated geographic areas and in vastly different societies, the earliest steps toward astronomy were taken in response to social needs. The regulation of social and agricultural activities by appropriate season, the establishment of festivals to honor deities that required propitiation turned men's eyes to the heavenly bodies from whose regularities time and the seasons could be fixed and religious calendars could be derived. It is interesting that the custodians of this kind of knowledge were members of priestly castes not only in Egypt and Mesopotamia, but also in Middle America where the life of the people was governed to an extraordinary extent by the cycles of Venus and other planets. The fact that in these diverse cultures the astronomers were exclusively priests also illustrates the way in which society affects the agents of science.

The development of metallurgy furnishes still another example of the way cultural or social pressure advances knowledge. The precise manner of the discovery of metal by Neolithic man is not certainly known to us. It may well have been, as has been suggested, an accidental one. Even its first use is conjectural. We do know that ornamental objects are among the earliest known artifacts in copper and bronze. But once the superiority of bronze over stone for tools and weapons was recognized, the obvious advantage it conferred on their users and on the possessors of the skill to make them brought on a rapid efflorescence of metallurgy. Tools and weapons that had been impossible or difficult to make in stone began to appear. And except for the use of stone for ceremonial implements, the new metal replaced the earlier material completely. There can be little doubt that the increased efficiency of these new tools created a cultural pressure that led to a rapid advance of metallurgical knowledge and skill. And the historic evidence of a later era is absolutely clear, that in a similar way the possessors of iron weapons enjoyed an incalculable military advantage over their enemies who still relied on bronze. To achieve such military advantages, society then as now would have exerted every effort to adopt and extend technological improvements.

These examples—and they could easily be multiplied—illustrate how social and cultural pressures may stimulate the development of the prototypic science of simpler stages of human society. The instances I have chosen emphasize the economic and the military. This is partly, I suppose, because the relationship here is most obvious and therefore occurred to me most readily. I am not prepared, consequently, without further inquiry to affirm that these are the principal or only channels through which social pressure on primitive science is exerted. It is useful, however, to remember that in primitive or simpler societies we can often see the reality of processes that are obscured in more complex ones.

If I may draw any inference from this brief survey of primitive societies, it would perhaps be something like this. The evidence from archaeology and from contemporary primitive groups suggests that science or technology constitutes an adaptive response to the environmental pressure exerted by society. There is some reason to think that competitive situations contribute to the rapidity of the response.

When we leave the earlier stages of human development and organization and enter into the highly complex structure of recent civilization, the relationship between science and society naturally becomes far more ramified and may indeed take on other and different aspects.

The following speakers will discuss three different examples of this relationship.

SOME REFLECTIONS ON THE STATE OF SCIENCE IN AMERICA DURING THE NINETEENTH CENTURY

By I. Bernard Cohen

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For Americans the outstanding problem in the history of science is to try to understand the failure of our country to produce a great scientific tradition in the nineteenth century. In presenting this problem, I have limited my discussion to the period from Franklin to Gibbs, two Americans who by their fundamental contributions to physics won the highest distinctions in science, such as foreign or honorary membership in the world's great scientific societies and academies—including both the Académie des Sciences (Paris) and the Royal Society of London. Between the two, the record in physics is meager. To be sure, we can always point with pride to Joseph Henry, but the fact remains that Henry was simply not in the same class with Faraday, Clerk Maxwell, Young, Fresnel, Ampère, Joule, Kelvin, Biot, Bunsen, Kirchhoff, Mayer, Helmholtz, and the other greatest giants of the nineteenth century in physics. Furthermore, if we seek mathematical creators in the same interval, there are the members of the Peirce family, but the only outstanding genius in mathematics in America in the nineteenth century was J. J. Sylvester, an Englishman who twice graced our shores. In astronomy things were a little better. There was Newcomb at the century's end and Pickering, and the early photographic experiments—the daguerrotype being used in conjunction with a telescope—and the work of Draper. But how paltry even this is by comparison with the great work done in Europe. In chemistry there are two contributions recorded in European annals: Hare's blowtorch and plunge battery and at the century's end the work of Josiah Parsons Cooke on the periodic classification of the chemical elements. In geology, America contributed more than in the abovementioned fields: at least descriptions of American geological formations made by native scientists were esteemed by Europeans. And the same is true of the biological sciences to an extent which may aggrandize the work of such botanists as John Torrey and Asa Gray all out of their due proportion. When all is said and done, however, so far as the great new ideas are concerned, or even ideas of somewhat lesser importance, the contributions of the nineteenth century made in America are few at best and enter the world's literature largely by courtesy. In one science only is it generally admitted that a major first-class contribution came from America—but, alas, the field is one which is not central to our understanding of the nature and interactions of matter, the creation and life of the universe as a whole, or the evolution of life and the basic nature of living functions. I refer, of course, to the work of Matthew Fontaine Maury, esteemed as a founder of the science of oceanography. One can have only the highest admiration for the brave pioneering work of William Beaumont, who made so much of an extraordinary opportunity to study the physiology of digestion. But the pitiful state of American science in his day can be seen in the enormous difficulties Beaumont encountered in attempting to do research and the general lack of appreciation of his magnificent achievements, which fortunately were esteemed in Europe.

Before exploring this period further, and inquiring into its relevance for our understanding of our own times, let me first clarify one or two major aspects of the

problem in the light of history. To begin with, we must face the question whether or not the production of men of science of the highest rank of genius is at all an index of scientific culture. We know so little about the biology of genius—its production and ecology—that perhaps it is not statistically meaningful to use data of this kind in characterizing our American culture. For let us suppose that there are twenty or thirty men of the highest scientific genius produced in the nineteenth century and that the countries where we might expect to find them include America, England, Scotland, Ireland, Spain, Portugal, Italy, Austria-Hungary, Germany, Russia, France, Belgium, Holland, Denmark, and Sweden. Taking as a weighting factor for each country its total population, or its number of enrolled college students, it may turn out that the American failure is not statistically significant. We would tend, therefore, to ask about a larger group, for instance the hundred or two hundred major scientists, the grade A men, not necessarily the A-plus: but the record would still be a poor one. Or, perhaps an even better index of our scientific culture might be the extent to which European scientists cited American scientific work—and here I mean not merely the descriptions of American fauna and flora or geological formations—or the magnitude of the European use of American scientific ideas. Pick up a European history of science in general, or any branch of science in particular, and we must ask whether the general omission of Americans can possibly be an act of ignorance or of mere national prejudice?

Historians usually couple the absence of great American science in the nineteenth century with the growth of America to the west and the rise of American industrial society. It is clear, I believe, and beyond argument, that in engineering, in the various applications of science, in inventiveness, America became a major nation early in the nineteenth century. Any comparative study of the number of inventions per capita in nineteenth-century America and other countries (by the crude method of counting patents) readily shows a greater number made by Americans than by those of any other nationality. Military historians often agree that the beginning of modern warfare occurred in the American Civil War; think of these innovations—iron-clad warships, fixed ammunition, naval guns with rifling, mass-produced uniforms and shoes, movement of troops by railroad, aerial observation, telegraph communication between headquarters and the front, photography, gas warfare, machine guns, and so on down a long list. No better index of American technological innovation could be found.

We must keep in mind that until almost the end of the nineteenth century, technology and engineering did not in general depend on the applications of pure science, by which I mean the discovery of new scientific principles. This was the era of empirical innovation and the time of mechanical invention. A typical major invention was barbed wire (Glidden's patent of 1874), based on a device for twisting two strands of wire with occasional barbed spurs. This wire made homesteading possible; in the great plains there was no supply of wood or stone for use in fencing off ploughed land to protect it from cattle. The reaper and sewing machine and the Colt revolver are other examples of this "mechanical" type of invention. Yet as early as the 1840's, the applications of scientific discovery were beginning to be of importance. As a type may I cite the telegraph and photography—both depending on recent discoveries in the pure sciences of physics and chemistry. From then

until now, with constant acceleration for about one hundred years, America has excelled in the applications of science—but not necessarily the science she has herself produced.

It is often assumed that the tradition in America has been one of engineering opposed to science, of applied rather than pure science. This point of view is misleading, not only because engineering had little to do with science during most of the eighteenth and nineteenth centuries; it is also wrong because in the Colonial period and well into the middle of the nineteenth century, our colleges trained no engineers. Until well into the 1840's, there was a conspicuous lack of interest in America in any form of engineering. When George Washington assumed command of the Continental army in Cambridge and surveyed his troops, he at once wrote a letter describing his two major needs: gunpowder (not then manufactured in America) and engineers "to supervise the works." Franklin's first two jobs on arriving in France were to get gunpowder and to recruit from the "corps de génie" some trained military engineers—chief among them were Louis DuPortail who laid out Valley Forge and was in large measure responsible for the plans at Yorktown. When the war was won, DuPortail drew up plans for a national academy of military engineering to be established at West Point. There was then founded the United States Military Philosophical Society, dedicated to the sciences of war and of peace, America's first national scientific academy. Both did not survive very long.

When the U.S. Military Academy at West Point was revived after 1812, it long remained the only training center for engineers of any sort in America. That explains why the railroads were all laid out by Army men: there weren't any other engineers in this country save for a few men trained in Europe. One American, Charles Storer Storrow, had the good fortune to be able to attend the Ecole Nationale des Ponts et Chaussées in Paris; his services were so much in demand as consultant for canal building, and the use of water for industrial purposes, that he quickly built one of the great American fortunes. America's first engineering school was Rensselaer Polytechnic Institute (founded in 1824). Then in 1848 there were founded Harvard's Lawrence Scientific School and Yale's Sheffield School. It is fair to say, therefore, that from about 1750 to about 1850 there was little or no interest in applied science and that only from 1850 to now could there have been the excessive interest in applied science said to be characteristic of America.

In eighteenth-century Colonial America the sciences flourished. The contributions made by Americans form a distinguished list. It was in America at this time that the first experiments on controlled plant hybridization were performed and the phenomena of dominance, xenia, and metaxenia recorded; later in the century the methods of detasseling corn used today were set forth clearly. It was in America that the first controlled test of the new method of inoculation for smallpox was tried out. Newton reported in his *Principia* that two Americans had produced splendid and reliable cometary observations. On our shores it was discovered that Northeast storms come from the Southwest. And it was in America that the modern theory of electricity was formulated and the law of conservation of charge applied to such phenomena as the condenser or capacitor. In all the Colonial colleges science was esteemed and encouraged, and formed an important part of the curriculum. The bills for purchase of scientific equipment in the eighteenth

century appear often to have been greater than in the nineteenth. When in the 1760's a great fire at Harvard destroyed the library and the "Philosophical Apparatus" the first steps taken were to replace the instruments of science. When the onset of the Revolution made it impossible for the senior class to do their experiments and observations, with the removal of the college from Cambridge, commencement was delayed so that no man would graduate who had not had opportunity to use the scientific instruments. I have looked carefully through the records of science in our Colonial colleges for any evidence of an organized anti-scientific spirit and have found none. Natural philosophy was generally held to be the friend and not the enemy of revealed truth; from the sermons of the Colonial divines one can easily make a compendium of the science of the times; and in every recommendation for a program of study for the ministry there is a recommendation for the study of science. As further evidence of scientific interest in Colonial America may I point to the fact that the second endowed professorship to be established in the New World is the Hollis Professorship of Mathematicks and Natural Philosophy (1723), that the first scientific society in the New World was founded in the seventeenth century, that in the eighteenth century two major scientific societies or academies were established and still flourish: the American Philosophical Society and the American Academy of Arts and Sciences. Finally, as evidence of the importance of pure science in those days, let us recall that for two days the Revolutionary War was suspended so that an expedition could be sent from the American side into British territory to observe a total eclipse of the sun. There were more Americans elected Fellows of the Royal Society of London in the eighteenth century than in the nineteenth. Even the debates on the Constitution of the United States were held up by questions of the meaning and possible applications of Newtonian physical principles. Could not one have therefore expected that the tradition so established would have continued and that in the nineteenth century there would have been even greater scientific movement?

For testimony on this point, may I refer to what is in my opinion still the best book on the science of the eighteenth century, Samuel Miller's *Brief Retrospect of the Eighteenth Century* (New York, 1803). Toward the end of the second volume, he discussed "nations lately become literary," primarily Russia, Germany, and the United States. He did not separate science and literature, but considered them together as cultural manifestations. In Russia Miller found an observatory, an academy of sciences, and schools for teaching science. He called attention to the high levels of geography, natural philosophy, mathematics, history, and medicine. Yet he ended on a melancholy note since learning was confined to a small part of the population while the great masses were illiterate. Germany was included in this chapter because the German language had not been used for literature and science until the eighteenth century. There were 15,000 authors active in that country, Miller reported, and about 6,000 new works published per annum; no one could obtain a higher degree from a university without publishing something. In Great Britain and Ireland he noted only seven universities, but in Germany there were 39 (of which six were created in the eighteenth century—including Göttingen, Erlangen, Bonn, and Stuttgart). Physical sciences, mathematics, and the biological sciences—all had flourished in Germany, while a new science had been created by Achenwall—*Statistik*.

Turning to America, Miller found the colleges generally good, but small. Encouraging was the growth of libraries. He noted the founding, not many years ago, of the first American medical schools. Clearly science had flourished to a greater extent than belles-lettres. Yet there were dangers ahead: (1) Most colleges, he observed, suffered from inadequate funds. Professors had to teach too many subjects; since they were not qualified for all, teaching was apt to become superficial and not respected. The trustees had contracted the time for the whole program so that "in some of these institutions mathematical science is unpopular, and the acquisition of as little as possible especially of the higher branches of it, enjoined on the student." (2) There was a general feeling that for men of learning the choice was between "erudition and poverty or comfortable affluence and moderate learning." No man had leisure to produce intellectual things. (3) Above all, he deplored the "want of encouragement to learning." Genius must be nourished by patronage, he said, as well as strengthened by culture. In the United States regard for literature or scientific distinction was small. "No rich fellowships exist in our universities to excite the ambition of students." Academic chairs, where they actually existed, had such small salaries that they did not tempt the prospective scholar. At the same time, the rewards of the state are small and its "favor capricious." Can it then be wondered, he asked, "that those who have some acquaintance with books, and hold important stations, are more anxious to secure pecuniary advantages, and to place themselves in a position independent of popular favor, than to make advances in literature, or to do honour to their country by the display of intellectual pre-eminence?"

Miller found "the spirit of our people is *commercial*. It has been said, and perhaps with some justice, that the *love of gain* peculiarly characterizes the inhabitants of the United States." Since the principal test of influence was amount of wealth, the acquiring of property naturally took precedence over letters and science, so that men of learning could expect "but little reward either of honour or emolument."

Although Miller ended on a more optimistic note, observing that "letters and science are becoming more important in the public estimation," the picture he painted was generally discouraging. And the next 50 years proved how right he had been. During the first half-century the older religious motives for studying science gradually withered away. To be sure, at many colleges science continued to be taught with religious connotations. Chemistry and the other sciences suffered somewhat in early nineteenth-century Princeton because of a fear that science was the enemy of revealed religion and the literal interpretations of Scripture, but toward mid-century this had passed and the faculty included such distinguished scientists as John Torrey and Joseph Henry, and there was pride taken in the announcement of the acquisition of a life-sized mannekin or *modèle d'homme* to be used in both the lectures on anatomy and on "natural theology." Young scientists and scholars at Harvard were told in 1847 that the connection between science and religion is not incidental or arbitrary, but inherent and essential. Both have as their materials the "thoughts of God," but religion "reads them in their source," and science "in their developments." "Brethren scholars, men of science, yours is a priestly calling."

It was, however, always hard to obtain funds for science, and apparatus was difficult to find in America. To illustrate the difficulties in the way of a man of science, let me take two illustrations from chemistry. When Silliman became professor of the subject at Yale, he found the need of special retorts with side-arms; the only one he had was broken at the joint. He sent his sample to a glass-maker in East Hartford who undertook to make him a dozen. When they arrived, Silliman opened the box carefully, to find that each retort was exactly like the sample, broken carefully at the joint, "like decapitated kings in their coffins." In 1816, an agreement was drawn up by J. F. Dana and Professor Gorham of Harvard in which Dana was to be paid $400 as assistant; the terms show us once more the difficulty in obtaining apparatus. Dana's chief duties were to prepare and demonstrate the experiments, then to clean and dry the glassware and return it to the shelves. The agreement stated that Dana was to be given permission "to use for his private experiments such vessels, materials, and instruments as, if broken or exhausted, may be replaced from Boston." But he was not to be allowed to use "the more valuable apparatus, tests, etc., which can be procured only in Europe."

In the 1840's Harvard could raise money to equip an observatory, but adequate funds could not easily be found for astronomical research. When John Quincy Adams rose in Congress to beg money for "light-houses in the sky," for observatories so as to put the U.S.A. in the same class with Russia, he was greeted with derision. It is well known that the attempts during about the first half of the nineteenth century to found a National Academy of Science met with dismal failure. And even after the present National Academy was founded during the Civil War, the remainder of the century saw it weak and without political influence. The Academy was not called upon to perform the major tasks for which it supposedly had been established.

Let us next examine the situation in American Science in the last decades of the nineteenth century. Consider first the case of Ira Remsen, the inaugural professor of chemistry at the first institution to be established in America for post-graduate work: the Johns Hopkins University. Remsen, be it observed, had been trained in Europe. He went to Munich in 1867 to study under Liebig, but Liebig at this time was taking no more students; Remsen then went to Göttingen to work under Fittig and Wöhler, receiving his Ph.D. in 1870. When Fittig went to Tübingen, he took Remsen with him as his assistant. Remsen returned to America and in 1872 became Professor at Williams College. There he found no laboratory. When he requested one, the reply of the president was: "You will please keep in mind that this is a college and not a technical school. The students who come here are not to be trained as chemists or geologists or physicists. They are to be taught the great fundamental truths of all sciences. The object aimed at is culture, not practical knowledge." Evidently, the nature of science or of science instruction was not well understood. Remsen was a persuasive man, however, and eventually he got a small laboratory. Here he continued the research for which he had been trained in Germany. The general atmosphere with regard to science may be seen in an anecdote which Remsen used to tell. He recalled a faculty meeting held in the college library. One of his articles had just been published in the *American Journal of Science*. Someone picked up the number, tried without success to read

the title aloud, and then followed some "good-natured fun." Said Remsen, "I felt that in the eyes of my colleagues I was rather a ridiculous subject." This story, typical of the period, indicates that research and knowledge were clearly not considered to be very important in American higher education.

For another view of science in the post-Civil War period, look for a moment at the career of J. Willard Gibbs. His life as a whole may be taken to prove that even if very few people in nineteenth-century America appreciated advances in the abstract sciences, Europeans nevertheless gave such work full recognition. Gibbs, who had been born in 1839, went to Yale College, and took his Ph.D. at Yale in 1863, the third year in which that degree was offered. He showed considerable ability in mathematics, and naturally enough in those days this led to a career in engineering. Gibbs wrote a thesis on the design of gears, invented a "center vent" hydraulic turbine, and also a railroad car brake (patented in 1868) which had some rather extraordinary features in that it worked when the train was going forward or backward and whether the locomotive was at the front or the rear end of the train. He also wrote a work on the governors of steam engines. It is perhaps characteristic of the times that when Gibbs was given the privilege accorded all men who had just completed their degrees of being allowed to teach, his job was to teach Latin; while his friend and fellow student Tracy Peck (later the head of the Latin Department at Yale) became tutor in mathematics.

Gibbs was not satisfied with his education and wanted to learn more, and went abroad to study. Note that he did not go abroad merely to become a scientist, but to learn more science. He studied in Paris and in Berlin, and he came under the influence of such men as Magnus, Weierstrass, Darboux, Chasles, Duhamel, and Liouville. The notebooks that Gibbs kept during this period show a shift in interest from governors and brakes to pure mathematics and mathematical physics. He returned to America, devoting himself to these latter two subjects, and his career was thenceforth marked by contributions to abstract or pure science, but not to engineering. In 1871 he became professor of mathematical physics at Yale, and it is another reflection on the times that such a man received no salary. He was offered a post first at Bowdoin in 1873, and later at Johns Hopkins in 1879, so that it cannot be said that he was wholly without reputation in America. J. J. Thomson later recalled the visit of a newly elected president of an American university who wanted to find a professor of molecular physics. Thomson recommended Willard Gibbs, only to be told that he probably had intended to mention Wolcott Gibbs, who was a chemist at Harvard (a very able man, though not in the same category as Willard). "No," replied J. J. Thomson, "I mean Willard Gibbs." The visitor asked for another name, saying: "Willard Gibbs can't be a man of much personal magnetism or I should have heard of him."

By 1886 the road was far easier for Theodore W. Richards than it had been for either Remsen or Gibbs. Richards was graduated from Harvard, where he had the opportunity to work under Josiah Parsons Cooke (1827–1894), who can fairly be described as the first university chemist to do truly distinguished work in the field of chemistry. Cooke was interested in the problem of determining the exact combining proportions of the various chemical elements, and he set T. W. Richards at the task of determining the atomic weight of oxygen in relation to copper. Richards soon was able to show that the accepted atomic weight of oxygen was too

high, that errors arose from the fact that nitrogen exists in most forms of copper oxide. A precocious scientist, Richards published two papers before he received his Ph.D. from Harvard at the age of 20. During the academic year 1888–1889, he had a traveling fellowship which took him abroad. There he came in contact with the men whose fame had reached him, who were esteemed for the high quality of their researches and their contributions to science. Stimulated by this experience, he returned to America to a teaching post at Harvard, determined to make his own contribution. In 1894, he visited Germany once again, and in 1901 he was offered a full professorship of chemistry at the University of Göttingen, which would seem to be the first time a German university offered a professorship to an American scientist. Within the span of these three generations—Remsen, Gibbs, Richards—American science came to its maturity, and it is perhaps both fitting and symbolic that Richards was the first American scientist to be awarded the Nobel Prize.

For a somewhat deeper view of this period, let us turn to the year 1872, when twenty-five frustrated American men of science asked the British physicist John Tyndall to come to America. The group was led by Joseph Henry and Edward Livingston Youmans, editor of the *Popular Science Monthly* and one of the great propagandists for science in America in this period. Tyndall had been invited to America on the specific ground that "a course of experimental lectures would materially promote scientific education" in the United States. Tyndall's fabulous success shows that there was a great interest in scientific subjects, even though nothing much had come of that interest by the way of advancing science. Tyndall gave six lectures in Boston, in Philadelphia, in Washington, in New York, and in Brooklyn; he gave two lectures in New Haven and three in Baltimore. After four months, he found himself the richer by $23,100, and was embarrassed to have made such a profit. At a banquet given in his honor before his departure, Tyndall announced that he was not going to take the money back to England with him, since the purpose of his visit had not been for his own pecuniary advantage. He therefore left the "surplus above expenses" in trust for the advancement of science in America, the interest to be expended "in aid of students who devote themselves to original researches." Many eminent physicists have been "Tyndall Fellows." The first was Michael Pupin, who has left for us in his autobiography, *From Immigrant to Inventor*, a dramatic and thrilling account of his adventures in becoming a scientist in America.

Tyndall said to Americans, "You are delighted, and with good reason, with your electric telegraphs, proud of your steam-engines and your factories, and charmed with the productions of photography. You see daily, with just elation, the creation of new forms of industry—new powers of adding to the wealth and comfort of society. Industrial England is heaving with forces tending to this end, and the pulse of industry beats still stronger in the United States." Yet it was at peril to the understanding that science was to be confused with industry. Science, said Tyndall, "must be cultivated for its own sake, for the pure love of truth, rather than for the applause or profit that it brings." He noted that no investigator of the first rank from the time of Aristotle to Stokes or Kirchhoff had a practical end in view. "They did not propose to themselves money as an end, and knowledge as a means of obtaining it. For the most part, they nobly reversed this process,

made knowledge their end, and such money as they possessed the means of obtaining it."[1]

Tyndall explained that three groups are needed for the health of society as regards science. First of all, there is required "the investigator of natural truth, whose vocation it is to pursue that truth, and extend the field of discovery for the truth's own sake, and without reference to practical ends." Then there is a group comprising the teacher, the public diffuser of the knowledge already won, the trainer of new scientists to come. Finally, there is the applier, the man who makes scientific knowledge available for the needs and the comforts of life. Alas, he pointed out, the popular notion of science in 1872 often related not to science as such, but to the applications of science. As he made clear, "such applications, especially on this [American] continent, are so astounding—they spread themselves so largely and umbrageously before the public eye—as to shut out from view those workers who are engaged in the quieter and profounder business of original investigation."

Tyndall reminded his hearers that in 1850 De Toqueville, in his book *De la démocratie en Amérique* had addressed himself to the problem of whether America would ever be a scientific nation. De Toqueville had said, "It must be confessed that, among the civilized peoples of our age, there are few [!] in which the sciences have made so little progress as in the United States." Had the United States been alone in the world, according to De Toqueville, Americans would have had to have made the discovery that progress in practical science cannot be had without cultivation of theoretical science.

But the Europeans had provided the theoretical science which the Americans were applying, and so the latter had never seen the need of adding themselves to the great store of science. "The future will prove," De Toqueville said, "whether the passion for profound knowledge, so rare and so fruitful, can be born and developed so readily in democratic societies as in aristocracies. As for me, I can hardly believe it." Among the reasons that De Toqueville adduced were the unquiet feverishness of democratic communities, the great excitement even in times of peace. There is in a democratic country like America, "attrition of man against man which troubles and distracts the mind without imparting to it either animation or elevation."

Here was a strong indictment. Tyndall, believing in the possible scientific future of America, found it necessary to reply to De Toqueville. If great scientific results are not achieved in America, he said, it will be owing to the fact "that the men among you who possess the endowments necessary for scientific inquiry are laden down with duties of administration or tuition so heavy as to be utterly incompatible with the continuous and tranquil meditation which original investigation demands." If "the original investigator had been honored as he ought to be in this land," he thundered at his audience, "I doubt if Henry would have been transformed into an administrator or Draper forsake science for history!" What was to be done? Tyndall made some interesting recommendations. In the physical sciences, he told his hearers, there is not a need for the "statical elements" of building so much as the "dynamical element" of brains. Use the existing institutions, he said, and let chairs be founded—sufficiently but not luxuriously endowed—"which shall have original research for their main object and ambition.

With such vital centres among you, all your establishments of education would feel their influence; without such centres, even your primary instruction will never flourish as it ought." He did not believe it would be fruitful to separate teaching from research, but he did feel that teaching ought to be subservient to research.

"Look jealously," he warned, "upon the investigator who is fond of wandering from his true vocation to appear on public platforms. The practice is absolutely destructive of original work of a high order." Now and then the research scientist might leave the laboratory for the platform for special purposes, particularly to tell of his own work, but generally it should be left to others. If this was so, was not he himself at fault for lecturing and on a public platform? Yes, he replied, but he had come for just such a special purpose. He had accepted the assignment to educate the American public, to explain the importance of scientific research and the nature of scientists.

Could we see the scientist at work, he said, the average man and woman would think them ridiculous. "To the uninitiated, they might well appear as big children playing with not very amusing toys." The object of attention of the scientist might seem "utterly trivial." But the "use" is not paramount—the scientist cannot ever "assure you that... [what he does] will put a dollar in the pocket of any human being now living or to come." Yet in the aggregate, science *does* put "dollars into the pockets of individuals," and "millions into the exchequers of nations."

In the wilderness, Tyndall agreed, the first job of man is to conquer the environment—as was true in Colonial times and as was still true at the time of his lectures (1872) in the West, where one would expect no science to be produced. But what of the vast portions of the American continent where man no longer battled with his environment—what of the centers in which he had lectured: New York, Brooklyn, Boston, Philadelphia, Baltimore, Washington, New Haven? Here he had found a love of science, in the soil prepared by Professor Youmans and others. He had evidence for this love of science when his audience came even in a blizzard. And they came not to hear about the "beauties" of science, but to listen to sober lectures on paramagnetism and diamagnetism, polarization, and the wave theory of light.

Reading the remarks made by Tyndall in 1872 is a sobering experience, as it is to follow the analysis of science and society made by Miller in 1802. And yet the historian cannot help wondering whether there is not another aspect of the failure of America to produce a giant in the realm of scientific thought in the era between Franklin and Gibbs. Undoubtedly in a society in which science (and intellectual culture generally) may be esteemed and strongly supported, a great many more students will be attracted to the life of scientific research than was the case in nineteenth-century America. Most likely, the probability of producing a Pasteur, a Darwin, a Bernard, a Helmholtz, or a Gauss may depend in some way on the number of active scientists in the land and the number of young scientists that they recruit to the profession. It is very likely more difficult to do productive research when one has to do too much teaching or administrative work, when one lacks funds to purchase apparatus and to hire assistants, and when one has to do outside

work to support the family. Yet for men of the very highest genius it is not at all clear that any such generalizations apply.

Despite the general indifference to research in pure science, Americans in the nineteenth century, after all, did a certain amount of respectable scientific work in a number of different fields. If these men were not Faradays, Liebigs, Lyells, or Müllers, the fault may have lain less with their environment than with their own characters and mental endowments. Consider the example of Asa Gray, one of the leaders of American science in the nineteenth century. Gray was an able systematist, trained as an M.D. and given a splendid introduction to botanical science by being research assistant to John Torrey. William Jackson Hooker considered Gray's first publication "among the most beautiful and useful works of the kind, that we are acquainted with." In 1838, after accepting a post at the University of Michigan, he went abroad and gained personal contact with many European botanists. He "created" the Harvard department of botany, was one of the founders of the National Academy of Sciences, and—according to his official biography—was a member of "sixty-six learned and scientific societies, ranging in degree from the Royal Society of London to the Polk County Agricultural Society in Iowa." His *Manual of the Botany of the Northern United States* (editio princeps 1848) is still used in modern revised form. Recipient of honorary degrees from Oxford, Cambridge, and Aberdeen, Gray achieved fame for his support of Darwin's theory of evolution, chiefly against the attacks of his Harvard colleague Louis Agassiz. Gray is apt to be remembered today because it was to him that Darwin wrote the famous letter of 5 September 1857 containing the statement of the theory of the evolution of species by natural selection—a letter published by Darwin to establish his priority of independent discovery.

Yet for all this, there is no place for Asa Gray among the very greatest scientists of the nineteenth century. Why did he not become one of the dominating figures in the history of scientific thought? He was well trained; he worked under Torrey, who was an able man; he went to Europe. In the Harvard community of those days there were eminent colleagues—among them Louis Agassiz, William Cranch Bond, Eben Norton Horsford. Science was then a more important part of the college curriculum than it is now, every Harvard student of the 1840's being required to devote at least one-fourth of the whole four-year curriculum to science and another one-sixteenth to mathematics. This requirement far exceeded the time devoted to Latin and Greek. The major lack, in modern terms, was a set of graduate and postdoctoral students. Gray had funds to build a botanical garden and herbarium, and yet he did not produce any of those grand generalizations that advance the science in one step far more than thousands of small paces of a lower order. His "greatest achievement, however, was his elaboration of the descriptive botany of North America," and perhaps equal to that was his training of so many eminent American botanists to carry on his work. If there were any limitations set on his career, so as to preclude his inventing any of the major scientific ideas of his age, these were not the effects of society so much as the qualities of his mind and personality. Gray's career seems to prove that the creative life of a scientist is controlled and directed by forces from within just as much as by forces from without.

We know so little about the effects of social environment on scientific creativity,

and are so ignorant of the biology of genius, that to make any generalizations on these topics is fraught with danger. Yet from history we may learn that the struggle to make Americans aware of the importance of pure or abstract science has been going on ever since the founding of the Republic. Those of us who sometimes become discouraged by the difficulties of educating the American public to the values of science may take heart from the noble line of those who have struggled before us. By the light of history, we may understand that many of the problems peculiar to America, in the relation of science to the public mind, derive from the relative youth of our scientific tradition. Keeping in mind the struggles of the past century, we may well doubt that there has been a permanent victory, that we may easily count on a general love of truth and respect for abstractions—even on the part of educated people—which would guarantee a public support of science for its own sake.

[1] The discussion of Tyndall's visit is based on John Tyndall: *Lectures on Light, Delivered in the United States in 1872–73*, with an appendix (New York: D. Appleton and Company, 1873), especially the Preface (pp. 3–6), the "Summary and Conclusion" following the last lecture (pp. 160–183), and the Appendix, containing Tyndall's remarks at Delmonico's, New York, 4 February 1873, at a banquet given in his honor.

*SCIENCE IN THE FRENCH REVOLUTION**

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Science presents itself to history under two aspects: first in its own evolution, and secondly, in its accommodation by culture. Which is the more important is a question of perspective, and which the more interesting a matter of taste. The evolution of scientific ideas relates the progress of science to nature, and is the more elegant and precise a subject. But its cultural history relates it to society, and it is that to which we are asked to address ourselves. I think it obvious that science, which is about nature, cannot be determined in its content by the social relations of scientists. At most it may be touched in style, in pace, and—within limits imposed by the logical interdependence of the sciences—in order of development. I should like to lay down, therefore, that I mean the term "French science" in the sense of scientific culture.

The Revolutionary torment in France was the birth agony of our democratic world. It drew science, along with everything of public moment, into its own great double movement. On the one hand, from the fall of the Bastille in 1789 to the fall of Robespierre in Thermidor, 1794, the tide moved irresistibly leftward to culminate in the Jacobin Dictatorship of the First Republic. This was messianic democracy, the most passionate attempt in history to realize moral ideals of virtue, of justice, equality and dignity, in political institutions, and to root them in nature. Once affairs were engrossed by Jacobinism, science was bound to incur enmity. This is not because scientists were reactionary or unpatriotic. On the contrary, they pressed into service on a scale unequalled until the twentieth century. But in its intrinsic combination of assurance and irrelevance, science

stood across the cosmic ideals of the Republic in a posture nonetheless insulting for being unpremeditated. Without ceasing to be science and becoming moral philosophy, it could not give the Republic the nature it needed, while the Republic to be true to its inspiration in the Enlightenment could ask no less. Thereafter—though on some matters simultaneously—the Revolutionary movement in its second phase was not so much back to the right, as forward—or inward—toward the integration of authority, the equalization of employments, the rationalization of institutions, and their summons to public service and (especially in the case of science) to education.

Not only was the French scientific community the most brilliant in the world, it was also the most highly institutionalized—a circumstance which permits us to be definite about its history. On the 8th of August 1793, in the late summer of defeat, alarm, and treason, and on the rising curve of the Terror, the Jacobin Convention abolished the learned academies as incompatible with a Republic. Counter-revolutionary writers always describe this measure as a simple act of intellectual vandalism. Moreover, there is reason to think that the Academy of Sciences, despite its healthy condition compared to the humanistic academies, was the primary target.

Three distinct themes recur in the campaign of slander which had gone before. First, these writings express resentment for the new chemistry, directed expressly against the person and the influence of Lavoisier, who appeared to his detractors, not in the humble guise of chemist, nor simply as a financier, but as the arrogant spokesman and evil genius of science. (In France much scholarly labor has been expended to show that Lavoisier was executed as a tax farmer. And I always think it a reassuring, though somewhat puzzling, implication that the wanton execution of a financier should be taken to present a less serious moral problem than the execution of a scientist. But the distinction is unreal. Lavoisier won odium, as he won reputation, both for his science and his public work).

Secondly, a gentler sentiment appears, which might seem inharmonious amid the muttering hostility of the *sans-culottes*. Enthusiasm for natural history was unanimous. The paradox, however, was only apparent. For the Revolution, which suppressed organized physics, provides institutional testimony to the deep instinct of romanticism to seek shelter in the humane metaphors of biology, which proposed organism rather than mechanism as the model of order. The Convention transformed the old *Jardin du Roi* into the modern *Muséum d'histoire naturelle*, and established twelve chairs of biological science. This was a truly munificent provision. It made possible the great age of comparative anatomy and the magnificent French tradition of experimental biology.

Finally, running through the attack of the Academy was a political assertion of the sort which rings of injured interests. Science was undemocratic in principle, not a liberating force of enlightenment, but a stubborn bastion of aristocracy, a tyranny of intellectual—and especially mathematical—pretension, stifling civic virtue and true productivity, drawing a veil of obscurity between nature and the people.

In objectifying, science alienates, and these were passionate outbreaks of that tension which must always exist between man's science and his desire to participate morally and through consciousness in the cosmic process. They were responses

loosed by social cataclysm, but conditioned by the terms in which Newtonian science had been presented to the Enlightenment. For the position was anomalous. Science basked and prospered, as everyone knows, in the atmosphere of its own prestige. But what is not appreciated, is how it was compromised by the deep commitment of the Enlightenment to the moralization of nature.

Particularly was this so at the heart of civilization, in France. For on the one hand, the Cartesian imperative to order and unity, confronted by all Newton's loose ends, was what carried the rational genius of France to the leadership of science. That critical instinct for mathematical clarity and theoretical elegance saved French science, humanitarian though it was, from submersion in the Baconianism which vulgarized the English tradition, and it was certainly this which was responsible for the poverty of British achievement in the abstract reaches of scientific thought after Newton. But if French science had its head in the celestial mechanics of Laplace, its footing was in the popular utilitarianism of the *Encyclopedia*, and this proved treacherous ground in the Revolution. For when that great undertaking is examined critically for its philosophy of nature, it appears less as an instance of Newtonianism, than as an attempt to draw its teeth and humanize it. In its theory of matter, in its enthusiasm for natural history, and in its half-Baconian, half-democratic sentimentality about humility and true knowledge in technology, that philosophy of nature presaged the pattern of events which engulfed the scientific community in the Revolution.

In the *Encyclopedia*, for example, chemistry is still subjective, an operational mode of communion with nature. It proposes to replenish the Newtonian destitution of matter by addressing itself to the sympathies in nature, the active principles at work in the world, not to the mass of bodies, but to their organization. Chemistry is intimacy with nature, the poor man's manual metaphysics, whereby that artisan, in whose skills true wisdom lies, manipulates reality, not in the humiliating abstractions of mathematics, but with his own hands: "Chemistry *unites* the two tongues—the popular and the scientific." This was the chemistry taught in popular courses, where pharmacists learned their art. In such eyes not only does Lavoisier insult and betray these hopes, but worse, the new nomenclature comes as a deliberate injury, making a mystery of the craftsman's livelihood, reducing him to dependence on the scientist.

This archaic chemistry of a world alive was congruent with Diderot's implicit conception of the entire *Encyclopedia*, as an immense substitution of organismic concepts and technology for Newtonianism at the center of science. For Diderot—and after him the whole romantic tradition—rejected the claim of mathematics to be the language of science. Geometry falsifies by depriving bodies of the qualities in which alone they exist for a sympathetic science. The mathematical spirit is worse than inhumane. It is arrogant, "prideful," the science of the infinite. But Diderot was no Pascal to agonize over infinity. He dismisses it, with perfect nonchalance, as uninteresting. Since knowledge must have bounds, let them be at the limits of the useful. And Diderot restores the mind to the coziness of a finite cosmos by wrapping science tight around humanity. In place of the image of matter in motion through the Euclidean void, Diderot—the advance guard for Goethe and the romantics—substitutes the model of the universe as a cosmic polyp: time, its life unfolding; space, its habitation; gradience, its structure; which embodies

the twin ideas of universal sensibility and evolution. Thus will biology supersede physics in a world we can fit. And Diderot uses a second metaphor: the swarm of bees. For the solidarity of the universe is social. On a cosmic scale, it is that community which the social insects know, and society becomes continuous with nature, the home of virtue, in an unbroken order.

In such a situation communication is direct, experiential. It does not lie through mathematics. It lies, instead, through craftsmanship, and so—as in Bacon—truth opens to the common touch, and right method dispenses the ordinary man from the need for genius. To the dignification of craftsmanship by science corresponds, therefore, a reciprocal democratization of science, one which entrained an inevitable cheapening of expectations. For this is the final consequence of the 18th-century humanitarianism which would retrieve from the cold abstractions of classical physics a science warming to man. It assimilates the whole of science to its applications, makes it only the rationalization of technology, and seeks in practice to obey Diderot's injunction to keep man at the center—not only man but every man. It proposes that dream of a citizen's science which the Revolution turned into actual measures.

All these ideas would never have broken out of the realm where such hopes and resentments always circle—except for the Revolution, when they provided the pattern in which statesmen responded to a political campaign against science, a campaign founded in real interests. It is obvious that the men who then came to power, their ears attuned to nature by Diderot and Rousseau, did not understand the conditions of scientific culture. The murmurs of 1789 were a chorus by 1793: Intellect is the enemy of liberty; erudition is unsuited to a Republic; Robespierre rejects Condorcet's proposal to base education on science, as tending toward an intellectual aristocracy, and prefers a Spartan education in civic virtue. And it is obvious that these misunderstandings arose from a peculiarly damaging moral enthusiasm for nature. For any glimpse of science itself could come only with the shock of betrayal of humane values, which—so the scientizing moralists had taught—derive through science from nature herself.

In this context, the Revolution gave the artisans of Paris their opportunity for a decisive offensive aimed at reversing the aphorism by which science governs the arts. A revolt of technology was one among the many rebellions swelling into Revolution. Specifically, the trouble went back to the Academy's responsibility for refereeing the monopolies and subsidies by which the State encouraged invention. This invidious role earned the Academy deep Gallic hatred among the artisans whose work it judged. These were not people whom it was safe to have offended, and the fall of the Academies set in train liquidation of the entire structure of French science. Rebellion was followed by purge. Direction of the metric project, for example, was assumed by a provisional committee composed of former academicians. One Prieur de la Côte-d'Or, a member of the governing Committee of Public Safety, sat for the regime and wielded its fearful authority. Like Carnot, he was a military engineer who had indulged vague scientific ambitions with no success. There he sat, as a colleague of Laplace, Lavoisier, and Lagrange, and he may not have felt at ease. On 23 December, they—together with Coulomb and Delambre—were removed by a decree of the Committee of Public Safety. The ground was that the state must assign missions only to men "worthy of confidence by their Republican

virtues and hatred of Kings." And from the acts of the regime, it is indeed possible to construct the Jacobin philosophy of science. It was clearly a forerunner of the Marxian, as was Jacobinism of Marxism. Theirs would be a science which, as to its technological aspect, would be a docile servant, and as to its conceptual, a simple extension of consciousness to nature, the seat of virtue, attainable by any instructed citizen through good will and moral insight. "Let anyone be a savant who wishes," is Cassini's summary of the Revolution—he had been ousted as Director of the Observatory by his research assistants—"O Happy Liberty."

Throughout, the spokesmen for science conducted its defense with neither skill nor dignity. One searches in vain for a single appreciation of science as a simple intellectual good. Instead, the Academy gave the case away and addressed vulgar Baconian platitudes about science and the trades to statesmen who were being told by the artisans themselves that science was throttling creative industry, and who had been taught by the *Encyclopedia* that it is the practical man who knows, not the theorist. Lavoisier and the others spared no efforts to draw off the menaces of the popular societies of technology: they even joined and sat endlessly on platforms listening to reports on silkworms and waterwheels, participating in proceedings which were a travesty of the Academy's own tradition. As the Academy's days ran out, and these bodies moved in for the kill, Lavoisier desperately wrote speeches to put in the mouths of sympathetic deputies—and then ran about the streets trying to find these few champions to send them to the Convention for some crucial vote. At the very moment of the Academy's dissolution, Lavoisier warned that if France abused the devotion of her scientists, she would lose their services. Nothing of the sort happened, of course. Reflecting on the behavior of the scientific community under the Revolutionary and Napoleonic regimes, one is reminded of the Schumpeter thesis about the bourgeoisie needing a master. As if to refute Lavoisier, indeed even as he moved ever closer to the guillotine, scientists were mobilized. They served brilliantly and performed prodigies in the famous effort of war production celebrated in every textbook. France did not lose their services. Only science did.

And yet, it is difficult to see how this mattered to science, deeply though it mattered to scientists. For a feature of the impersonality of science is that it does emerge from all the vicissitudes it inspires. Even the terrible tragedy of Lavoisier was, after all, personal. It is fair to say that he had already made his contribution to science. Before ever he fell victim to the Revolution, he already had begun to fall incurably victim to that subtler fatality which turns the creative scientist into a statesman of science, into a public figure.

Moreover, the liquidation of the science of the old regime is only one aspect of its story in the Revolution. The other, the rationalist tradition of scientific interpretation, though temporarily overwhelmed, had too its origin in the Enlightenment, and came into its own after the fall of Robespierre. This was the tradition, after Locke, of the associationist—or the positive—psychology, according to which man is what he makes of his experience. It would base society, not on virtue, but on talent. It was the tradition, too, in which scientists—as distinct from philosophers—themselves participated: D'Alembert, Lavoisier, Condorcet. And after Thermidor the authorities hastened to make amends. Upon the *tabula rasa* left by the Jacobins they erected a new set of scientific institutions: *écoles*

centrales, the *École normale* of the Year III, the *École polytechnique*, the medical faculties of Paris, Strasbourg, and Montpellier, the *Conservatoire des Arts et Métiers*. Only the *Muséum d'historie naturelle* emerged flourishing from the Terror. Other schools were revived: the *École des mines*, the *École des ponts et chaussées*, and the *Collège de France*. Finally, at the summit was created the *Institut de France*. Thus, France was endowed at one stroke with her scientific institutions, and the first generation who taught and studied in them assured the restoration of her scientific leadership and its enlargement through the early 19th century.

It was a remarkable effort, animated by a consistent philosophy, which was nothing less than to unify the sciences through a common conception of the nature of scientific explanation, and in so doing to link them, both institutionally and philosophically, to realization of the idea of progress. So for a time science was conceived as a function of its educational mission. The *École normale* assembled the first scientific faculty, to be taken over by *Polytechnique*, as distinguished a faculty, man for man, as has ever existed. For the first time, students were offered systematic technical instruction, directed toward engineering, to be sure, but on a high theoretical plane, and under the foremost men: Lagrange, Laplace, Monge, Prony, Berthollet, Guyton, Chaptal. The students, able and eager, chosen by competition, were immensely exhilarated by the sense of being conducted at once to the very forefront of scientific conquest, and at being told that the future of the Republic, which is to say mankind, depended on how they acquitted themselves in so exposed a situation.

But *Polytechnique* had an equal influence on its teachers. If one were to read only the research memoirs of the Institute in its first ten years—up to 1805— and compare them to those of the old regime, one would conclude that French science had gone down with its Academy. Quite erroneously—for the explanation is that scientists were communicating, not primarily with their colleagues, but with their students. *Polytechnique* made scientists into professors—again for the first time. It brought Laplace back to Paris from the refuge he had taken in 1793. It brought Lagrange back to mathematics from the preoccupation which had enveloped him since completing *Mécanique analytique* ten years before. Monge drew his descriptive geometry together only for his course. So, too, did Laplace come to write the *Système du Monde* and the *Essay on Probability*. Cuvier's *Lessons on Comparative Anatomy* were given at the *Collège de France*. Lamarck first presented the idea of evolution as the framework for his lectures at the Museum. In short, the systematic treatise displaced the research memoir for a time.

It was the necessity to reorganize science for teaching which produced this general movement of rationalization. In the philosophy of science, this movement takes its place between the Enlightenment and positivism, introducing a displacement toward action, a great enrichment of detail, and a certain access of rigor. It involved all the sciences in a common preoccupation with method, with classification, with nomenclature. The author of a treatise, at once investigator and professor, would address himself to his entire science, which he would expound according to whatever principles resolved it into a rational body of knowledge. He would present them, not just as an authority, but argumentatively, as an advocate. His claim to originality lay, less in this or that discovery, than in having discerned the principles. So Cuvier founds the method of comparative anatomy

in the principle of subordination of parts. So Berthollet does not just state a Law of Mass Action—he makes it (unfortunately, as it happens) the principle which will rationalize chemistry according to affinities in the circumstances of reaction. I cannot, of course, undertake a rehearsal of all the scientific literature. But it is astonishing how nearly all the titles are variations on a single theme—for example, Prony's "*Philosophical* Mechanics—or *Rational Analysis* of the Different *Parts* of the Science of Equilibrium and Movement." "Philosophical" and "Analytical" are the key words—for underlying them all was an implicit conception of scientific explanation as a kind of cosmic education.

In this light science itself is positive knowledge, of course. It is its *function* in the world which is educational (or philosophical—the words are 18th-century synonyms) and its mode of procedure which is analytical. First science seeks to discern the elements of a complex subject. These once found, it ranges and classifies them according to the logic of nature underneath the welter of phenomena. Next, it establishes a systematic nomenclature designed to fix the thing in the name, cement the memory to nature, and fasten the idea to its object. So the human understanding will be led toward a rational command over every department of nature by following its inherent order. Scientific explanation, then, consists in resolving a subject into its elements in the objective world, in order to reassemble its images in the mind according to the principles of the associationist psychology. The inspiration was algebra. But the model was botany.

In fact, of course, this confidence in the universality of analytic method rested on a semantic illusion. What is an algebraic process in Lagrange is simple taxonomy among the naturalists. But the difficulty was concealed by a very worthy commitment to the unity of science: to that kind of tolerance and mutual respect which rescues communication from specialization by means of the comparative approach. So Monge gave credit to Linnaeus for his idea of grouping surfaces into natural families. So Bichat brought anatomy to the instruction of physiology by founding histology in the classification of tissue. So Berthollet looked to statics for the idea of chemical masses in equilibrium. It was Lagrange who had suggested to Lavoisier that the future of chemistry lay in turning it into a material algebra, and this truly was Lavoisier's inspiration.

The force and range of the work were remarkable, then. I do not think it would be an exaggeration to call this French essay in rationalization the last thrust of the Enlightenment, by which the Enlightenment returned whence it had originated, and repaid the debt it had incurred to scientific culture a century before. It imparted a unity to scientific effort which it was not to know again. Not only so, but it may be that this is the last phase in the history of science which belongs to humanism. For as a matter of principle, no one of these savants limited his vision by his science. "It is certainly," wrote Cabanis (who founded moral philosophy in physiology), "a magnificent and beautiful conception, to consider all the arts and all the sciences as forming a community, an indivisible whole, limbs from the same great trunk, united by a common origin, and still more by the fruit they are destined to bear: the progress and happiness of mankind."

* This paper is the abstract of a book on the subject which the author has in preparation and which will carry, of course, a complete documentation of the argument. In the meantime a much fuller account of the Jacobin attack on science was presented before the University of Wisconsin-

National Science Foundation Institute of the History of Science in September, 1957. That paper, "The *Encyclopédie* and the Jacobin Philosophy of Science," is in press in the symposium which records the acts of the Institute, Marshall Clagett, ed., *Critical Problems in the History of Science* (Madison: University of Wisconsin Press, 1959), to which the reader may turn for a preliminary (though considerable) documentation. To reproduce the annotation here would burden this short paper very heavily indeed.

SOCIETY, SCIENCE, AND EDUCATION IN THE SOVIET UNION

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In the summer of 1957 while in the Soviet Union as a tourist, I was invited to address one of the institutes of the Academy of Sciences of the USSR on the organization of scientific work in geography in the United States. Many times I had been asked about the American national plan for scientific research, how it was directed, who determined on which topics research would be undertaken, how scientific research was supported, and similar questions. On that occasion I felt obliged to explain that the wide diffusion of responsibility for scientific research and education in the United States reflected the social, economic, and political system of this country and the history of its development.[1] One needs to recall that the American Revolution of 1776 was characterized by serious misgiving about the dangers of unchecked centralized government power. As a result, in the United States political responsibility and power is divided among the executive, legislative, and judicial branches of government at the federal level, and among federal, state, and local governmental units. Furthermore, some aspects of society are held altogether outside the domain of government. The program of higher education is divided between publicly supported universities of 48 separate states and of other government units on one hand, and private universities of many types on the other. Scientific research is pursued independently by universities, government, and industry.

Now in the reverse situation I must try to make clear that the organization of scientific research in the Soviet Union has also been profoundly affected by a revolution, more recent than the American one and of a very different character.

The Communist Revolution, in sharp contrast to the American, was developed on the basis of great faith in the power of government to transform society for the betterment of man. The Communists seized the government not to restrict its power but rather to use it. They have aspired fundamentally to remold society through a state monopoly of the production of goods, of means of communications, of education, and of science. Thus, whereas much of the support for science in the United States comes through private or state universities, through individual interests, or through great corporations, in the Soviet Union support comes from the state and is directed in accordance with comprehensive state plans and policies. The state is not the last resort in solving economic and social problems, it is the basic agency with a monopoly of power.

Modernization in the Soviet Union.—Some light may be shed on the nature and

interrelations of Soviet society, science, and education if one considers the Soviet Union as a country in the process of rapid modernization. Because of a different historical background in Russia, later timing, and dissimilar leadership and philosophy, the modernization is developing along lines quite different from those followed in Western Europe or the United States. In many ways Russia had lagged behind Western Europe, as symbolized by the persistence of the old-style calendar. If I may be somewhat impressionistic, I should say that four great revolutions which had long since shaken Western Europe were overdue in Russia at the beginning of the Twentieth Century. When they arrived, they came not singly but concurrently with agonizing suddenness, intensity, and depth. I refer to the religious revolution embraced in the West as the Reformation and Counter-Reformation, the economic revolution which broke forth in England as the Industrial Revolution, the political revolution which found expression in the movement toward democracy in the French and American revolutions, and the intellectual revolution, called the Age of Reason or the Age of Enlightenment. The first of the revolutions took place in Western Europe in the 16th and 17th centuries; the second in Britain in the late 18th century; and the third in France and America in the late 18th century; and the fourth throughout the Western World in the 18th century. These great movements which required centuries of gradual evolution in the West were telescoped into a few decades in Russia and have occurred simultaneously during the lifetime of the present generation. No wonder the complexly intertwined effects of these four revolutions have been difficult to unravel!

The Reformation and the Counter-Reformation of the 16th and 17th centuries took the form of Protestantism in Britain and Northern Europe and of a cleansing and revitalization of the Roman Catholic Church in Southern Europe. Although there had been minor adjustments and some modernization in Russia, the Russian Eastern Orthodox Church had not been subject to the searching reforms produced in the West by the Reformation. The Reformation, arriving four centuries late, exploded violently in virtual destruction of the old church and its replacement by Communist atheism and dialectical materialism, which has been established as the state religion of the Soviet Union, compulsory for members of the Communist party who, in a sense, form the priesthood of the new order. It is suggestive of a recognition of man's deep-seated psychological, emotional, and spiritual needs that the Communist Party, consciously or unconsciously, has appropriated many of the forms of the church in an attempt to capture emotional loyalties, reverence, sense of security, and abiding faith of the individual in a greater power outside himself. The theology and moral teaching of the Church have been replaced by the equally inclusive ideology of communism. Communism has its dogma authoritatively interpreted by the appropriate spokesmen. It has its scriptures in the writings of Marx, Engels, Lenin, and formerly Stalin. Some Soviet writings resemble sermons, with appropriate quotations from each of the revered "fathers." The tombs of "saints" Lenin and Stalin are objects of pilgrimages by the faithful; one of the Intourist guides in Moscow wept in the Lenin Museum out of a sense of religious exaltation at being on such holy ground. This new state religion has its devoted and enthusiastic missionaries who attempt to convert others to the new faith. Sadly enough, it also has its history of inquisitions, witch hunts, and denunciation and execution of heretics. One of my strongest impressions of the Soviet Union was

the sense of being in the presence of a great religious transformation, imposed by the will of the party leaders through the complex machinery of the party, education, scientific popularization, police, and state, but widely accepted by the younger generation.

The relevance of this revolution to work in science is as follows: (1) the top scientific administrators are chosen in large numbers from active members of the Communist Party, (2) most scientists are expected to conform to the faith, to avoid heresy or heterodoxy, or at least to be discreetly silent if not active in support, and (3) scientists trained from youth up to accept the superiority of the system as a matter of religious faith and patriotic pride tend emotionally to accept the value of the system as a whole and to direct any critical comments toward only the very narrow segments of the society with which they may have direct contact.

Perhaps the mention of the name of T. D. Lysenko will evoke some of the implications and dangers of the application of this modern dogma to science. Eric Ashby's delightful and insightful account of science in the Soviet Union contains a very perceptive analysis of Lysenko.[2] Lysenko's highest authorities for the science of genetics are the saints of dialectical materialism, Marx, Engels, Lenin, Stalin, and Michurin. Heretics in his view are to be indicted and heresies utterly annihilated. His writings are an odd mixture of rational argumentation and of the emotional fire and vision of the zealot denouncing sinners and heretics, with such name calling as "idealism" for Kant, of "formalism" for Mendel or Weismann, of "capitalistic" for Western scientists, of "priestly" for Mendel, of "fascist" for anyone who sees an innate heterogeneity of plants, animals, or people, and of "abiological" for those who call for statistical evaluation of reported experiments. If experiments conflict with higher political authority, the higher authority must prevail. I trust this case is striking enough to make clear my point that in the Soviet Union the rise of a religiously cultivated atheism has not eliminated the problem of dogma and emotionalism which plagued science in the West and in Russia for many centuries. Just as the 19th century Russian scientists did not publish direct attacks on the tenets of the Church so the present scientists dare not directly confront the contemporary dogma.

Vacillation between contradictory positions has long characterized Russian history: between looking toward the West, as under Peter the Great, or turning the back on the West, as has been more common. Intense mystical and religious nationalism, which rejects the universality and objectivity of science, is like a volcano in having periods of quiescence then of acute activity. The last years of Stalin represented a period of intensity. Recently Soviet leaders have turned again to the international aspects of science; for scientists in all other parts of the world this is a happier and more hopeful phase.

The coming of the second of the revolutions, the industrial revolution, has taken the form of state ownership and management of the entire economy. Under it the Soviet Union has wrought a massive modernization and rapid industrialization with emphasis on heavy industry. The forced growth of manufacturing has been paralleled by an explosion in the needs for trained engineers and scientists.

The coming of the third revolution, the democratic political revolution, has found expression in widespread participation in the forms if not the actual power of political selection, in universal participation in the process of education, and in

popular adult education. Education has been brought to the masses. The training of scientists has reached into all segments of society for able youngsters capable of a scientific career. In channeling exceptionally talented individuals into scientific training Soviet society appears to have reached a high degree of efficiency.

The streams of intellectual thought that found expression in the Age of Reason with figures such as Voltaire in France and Benjamin Franklin and Thomas Jefferson in America also flowed into the thought of the Soviet Union. Faith developed in the perfectibility of man and of social institutions, in the ability of man by rational thought and by scientific investigation to improve himself and society and to rule the universe. In the Soviet Union this revolution took the form of an optimistic faith in the ability of the Communist Party and the government, through the instrumentality of science and industrialization, to create a rational society, a Utopia in this world.

According to the Soviet view, Soviet society is based on science and the evolution of society can be scientifically planned and controlled. In theory at least science rules supreme and nothing is allowed to interfere with the progress of science. In practice the final arbiter of what is acceptable in science may not be the scientist himself but the political leader at any given time as indicated by Stalin's authoritative pronouncements in linguistics or by Molotov's dogmatic determination of scientific truth in the field of genetics.

The philosophy underlying a gigantic investment in science and education is the belief that they are productive forces and that funds spent in each, just as money expended in the construction of factories, constitute a capital investment making possible a greater total national production.

Science is supported not out of mere intellectual curiosity but as a tool for the mastery by man of physical resources to the end that their utilization for the production of goods may increase the total material and military strength of the country and the standard of living of the people. Science is not a philanthropy or a field of intellectual delights but a productive capital investment.

The government and the party in the Soviet Union maintain a monopoly over the key sectors of the economy, including science. It is felt that the government has not only the right to the results of education and scientific research but also the obligation for the full support of these institutions. Science is too important to be left in private hands. The state assumes full responsibility for financing the training of scientists, for their assignment to positions where needed, and for the support of approved and needed research.

Centralization in the support and control of science has had both strengths and weaknesses.

The strengths are: (1) abundant financial support making possible rapid growth in the number of trained scientists and a tremendous development of research and educational institutions; (2) coordination of programs of training scientists and of their utilization in research institutions, in universities, and in government; (3) ability to focus scientific resources on fields of science that appear to hold most promise in terms of the over-all policies and needs of the state, as, for example, nuclear, electronic, or rocket research on one side or resource analysis for the successive five-year plans for economic growth on the other.

The weaknesses of the system are: (1) fields of great potential importance may

be neglected because the research planners see no practical or scientific interest in them; centralized decisions avoid diffusion of effort but also lack the safety valve of diversity of judgment and personal interest of the individual scientist; (2) political and dogmatic considerations may interfere with freedom of scientific inquiry or interpretation; (3) practical and short-range research at times may tend to be developed to the neglect of basic research.

Perhaps part of the attractiveness of a career in science in the Soviet Union is not the result of planning but occurs in spite of it. Natural science provides a relatively safe refuge from political dogma and from dangerous and unpredictable ideological shifts. Two pieces of evidence support this view. In the first place, the basic middle school textbook in physics has gone several years without substantial change, whereas the history textbook has suffered frequent radical revisions as the party line has shifted. Thus the violent changes after the death of Stalin directly affected every person teaching or working in the social sciences or history but left most of science untouched. A second piece of evidence is that in institutions of higher learning in the Soviet Union a lower percentage of scientists are members of the Communist Party than faculty members in other fields. The extreme range is from the faculty in the physical-mathematical fields with only one-fourth being party members to the so-called social, political, and philosophical fields with over 90 per cent.[3] Absence of party membership does not necessarily indicate opposition to the party, or even indifference; it may be evidence of lack of time as party duties may require many hours a day of essentially political activity and a dedicated scientist may not care to spend that much time away from his scientific work.

Nevertheless, at the top level in the Soviet Union there appears to be a close tie-in of scientists with party and government administration. For example, two of the scientists with whom I talked on my recent trip were members both of the Academy of Sciences and of the Supreme Soviet, which, with some notable differences, corresponds roughly to the American Congress. This is a two-way street. The voice of scientists is heard in top government circles but in the reverse direction political considerations may penetrate scientific work.

The Academy of Sciences of the USSR.—The key scientific organization in the Soviet Union is the Academy of Sciences, which is not just a coordinating agency but is itself the largest research institution of the country, perhaps of the world. The Academy of Sciences is, in effect, the ministry for scientific research of the government. As such it has a duty to fulfill the specific tasks assigned to it by the government. It is entrusted with the important duty of coordinating and harmonizing scientific work among its own institutes, other institutes, regional academies of science, and the increasing research programs of the universities.

The Academy has 8 major divisions and 120 separate institutes and branches.

The eight major divisions are (1) physics and mathematics, (2) chemistry, (3) biological sciences, (4) technical sciences, that is engineering, (5) economics, philosophy, and law, (6) languages and literature, (7) history, and (8) geology and geography.

The Division of Geology and Geography, to take one example, has some 16 major institutes or institutions under its jurisdiction. I am most familiar with one of the institutes within this division: the Institute of Geography, one of the 120 institutes of the Academy. This one institute now has a full-time research staff

of more than 300. It is doubtless the largest institute under one roof anywhere in the world devoted entirely to scientific research in geography. The institute is of particular interest in that it embraces elements of the whole scholarly spectrum from the physical and biological sciences into the social sciences. It consists of ten sections, each with about 30 scientific workers as follows: general physical geography, geomorphology, glaciology, climatology and hydrology, biogeography, cartography, history of geography, economics geography of the USSR, geography of the peoples democracies, and geography of capitalist countries. The institute maintains four specialized laboratories in Moscow and four field research stations: in the Arctic, in Central Asia, in the Urals, and near Moscow. I spent several days at the Institute and was impressed by the seriousness and ability of its scientific research personnel.

The Institute of Scientific Information of the Academy of Sciences now produces a series of bibliographical and abstract journals. The one on chemistry is said to be less complete than *Chemical Abstracts* but the one in geography is far more detailed than any bibliographical or abstract publication issued in the Western world.

A major effort is made to bring science to the people in a series of popular lectures. Great scientists are strongly encouraged to give such popular talks. The All-Union Geographical Society, for example, has an extensive program of such popular lectures, either at the Society or in schools and factories.

Perhaps it should be mentioned that the Academy of Sciences has also had its periods of strain and purges. As reported by Vucinich, there have been three periods of crises: (1) in 1929–1931 when pressures were applied to the older non-Communist members of the Academy and perhaps 10 per cent of them were removed; (2) 1936–1938 when the Communist members were caught in ideological and power shifts and perhaps half of them lost; (3) 1946–1953 in a complex move against possible internationalization, for increased applied work, and for planned great projects rather than individual research.[4] This last crisis ended with Stalin's death.

Education.—Soviet education clearly has two major aims: (1) to inculcate a deep and abiding faith in the leadership of the Communist party and in the superiority of planned communist society over all other forms; and (2) to develop the maximum productivity of individuals in designated occupations.[5]

Soviet education has grown rapidly in recent decades. There are now 15 times as many students in institutions of higher learning as there were forty years ago. Illiteracy, said to have amounted to 80 per cent forty years ago, is now virtually wiped out. Universal primary education has been established among all peoples. Universal compulsory secondary education is being extended to all cities. Education and science are on the march. The Soviets are very proud of this progress and rightly so. Refugee interviews reveal that though former Soviet citizens have loud complaints about some features of Soviet society, such as the power of the secret police and the forced collectivization of the farms, they feel that in the development of education and science the regime is worthy of praise and support.

The educational system is centrally planned with respect to number of students, allocation of students among types of schools, among fields of study, and, on graduation, among positions. All aspects of school life are planned. The textbooks are

uniform throughout the Russian-speaking portion of the country. Students who have been through the same grades in the same type of school have had identical courses.

The Soviet middle school just now is in a state of tension and transition. As late as 1950 nearly all graduates of the ten-year school continued their study in institutions of higher learning. The decision to move toward universal secondary education in the cities has resulted in a very rapid increase in the number of students in the last three years of the ten-year school, which corresponds roughly to the American high school. The number of students in these grades multiplied six times in the six years, 1950–1956, whereas the number entering institutions of higher learning remained about the same. As a result in 1956 only a sixth of the students completing the ten-year school continued into higher education.[6] The curriculum of the ten-year school, designed as preparation for further academic work, is not considered practical enough for the students moving directly into jobs at the end of school. The Soviet Union is now being faced with the same conflicts that have plagued the American high school in the reconciliation of high standards for gifted students preparing for further careers in science and scholarship with the practical needs of larger numbers terminating at this stage of education.

In one respect Soviet education is very similar to American: in its democratic character and scope. The number of students in institutions of higher learning in the United States is now approaching three millions and in the Soviet Union two millions. Of all such students in the entire world, the United States has about a third, and the Soviet Union about a fourth. According to a recent UNESCO handbook these two countries together have 57 per cent of such students in the entire world.[7] These figures take no account of enormous differences in the type, purpose, or quality of higher education in various countries.

The Soviet Union has placed main emphasis on applied technology. In most institutions of higher learning, it is possible to specialize only in some applied field such as agriculture, railroad transport, mechanical engineering, geological exploration, or teaching. Among the more than 700 institutions of higher learning, science majors as such appear to be available only in the 36 universities, which account for only about 10 per cent of the resident enrollment of institutions of higher learning. Within the universities proper, however, the sciences are overwhelmingly dominant. More than half the university students major in the sciences; about a fourth in physics and mathematics alone. Furthermore, three-fourths of the university advanced degrees are in the natural sciences. In terms of numbers of university students the principal fields of science in order are: physics and mathematics, geology, chemistry, biology, and geography. Outside the sciences the largest number of university students are in languages.

One of the major characteristics of Soviet training in science is the emphasis on the study of a foreign language, usually English, as a tool for scientific work. By the time a scientist has achieved the training equivalent to an American Ph.D., he will have completed six years of language in the school, four in the undergraduate course in the university, and one in post-graduate work, or a total of eleven years. As a result Soviet scientists in general read English with facility but lack opportunity for hearing or speaking it.

The University of Moscow serves as a symbol of the seriousness with which

society, through the government, takes its responsibility for the training of future scientists and scholars. The university is an international showpiece. Its new campus on Lenin Hills, devoted to the sciences, spreads over more than a square mile and contains 83 large buildings. The central building is 786 feet high; that is really higher education! The equipment alone for this new science campus cost over one billion rubles, or more than one hundred million dollars.

Three features of education for science impressed me particularly.

First, the state takes financial responsibility for the training of scientists, from the beginning of the elementary school through the university. Tuition is now free, having been abolished in the middle schools (classes 8 through 10) in 1956. State financial responsibility includes not only the institutional costs of buildings, equipment, supplies, teachers' salaries, libraries, and laboratories, but also the expenses of the students in the university. Scholarships are for the costs of living in the university dormitories, for food, and books. A majority of the Moscow University students live in the dormitories. Eighty-six per cent of these students have scholarships; 14 per cent either do not need them or cannot qualify.

Second, loss by failure is incredibly low; only 6 per cent of the entering students at Moscow fail to graduate five years later. Several factors may account for this. (1) Good selection on the basis of previous work. (2) Studies being similar to jobs, students do not move at will. (3) Attendance at all lectures and examinations is compulsory. (4) Because of urgent needs for the trained personnel, the university may have quotas to be met on the number to graduate.

A third impressive aspect of training is the smooth flow of graduates into appropriate positions. A commission simply assigns students to jobs. The commission may take into account student preferences as well as requests from possible employers. The most able students are regularly assigned to research in institutes of the Academy of Sciences. It is said that the poorest students are assigned to teaching in the schools.

The training program seems to me to have some weaknesses also.

First, the lecture load is excessive. In universities freshmen are required to attend 32 hours of lectures each week.

Second, the undergraduate program seems to me excessively narrow. Students do not explore intellectually. They learn almost nothing outside their own speciality except certain necessary tool subjects and a bit of Marxism-Leninism.

Soviet education seems to me good for professional work but poor for a rich life.

In my visits to the University of Moscow I had time for a detailed study of only one of the divisions of the university, the Faculty of Geography, the staff of which numbers 482. In terms of size of staff, amount of space, and quality and quantity of equipment available, the Faculty of Geography at Moscow University exceeds any of the departments of geography in the universities of Western Europe or the United States. The University of Moscow is also a major center of research. For example, 60 staff members in geography are supported directly by contract research for various governmental agencies.

Only about 3 per cent of the graduates of institutions of higher learning proceed on to graduate work. Whereas the loss of students in undergraduate programs is very low, losses among graduate students are very high. Many drop out during

the courses; others never finish the dissertations; and most take much longer than the program is planned to require. Apparently the transition from the rigidity of a set lecture program to the flexibility and freedom of independent scholarship and research is difficult.

In the Soviet Union scientists and professors have both high status and high relative income. Based on a tourist rate of exchange of 10 rubles to the dollar, professors who are department heads receive $7,000 per year. Farmers on the collective farm I visted near Rostov received last year $700, or just one-tenth as much. Half of the farmer's income was paid in grain, butter, or hay for his own use. Young workers in a shoe factory I visited in Kiev received $840 a year for a 46-hour week. Some laborers are reported to receive as little as $350 a year. Relative to other individuals in Soviet society, the professor and the scientist occupy an extremely favorable position.

Summary.—The evidence seems clear that the Soviet Union has succeeded admirably in training and productively utilizing a very large number of scientists, that it has been able to achieve high levels of scientific effort in many fields, and that it has been able strongly to motivate scientists by a system of high financial rewards, high social status, and appeals to patriotism and social responsibility as well as to scientific curiosity.

[1] Harris, C. D., "Geograficheskie Issledovaniia v S.Sh.A." (Geographic Research in the United States), *Vestnik Akademii Nauk SSSR, 28*, No. 2, 23–30 (1958).

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[3] Korol, Alexander G., *Soviet Education for Science and Technology* (New York: Wiley, 1957), p. 292.

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*METABOLIC ACTIVITIES OF SUBCELLULAR PARTICLES FROM AZOTOBACTER VINELANDII**

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The subcellular fraction of bacteria referred to as "protoplasts" retains a large amount of the activity of the cell.† For nitrogen-fixing species, the ability to incorporate nitrogen by this fraction would seem to be a prerequisite for its appearance in further purified fractions. Merely diluting such protoplasts yields a par-

ticulate preparation which may answer the need stated by Magee and Burris:[1] "A more gentle (and yet complete) method for cell rupture would be highly desirable, and it would be valuable to test cellular fragments more extensively for their ability to fix nitrogen, as the enzyme system may be closely associated with the cellular membrane. . ."

The object of this study is to investigate the enzymatic properties associated with nitrogen fixation of protoplasts prepared from *Azotobacter vinelandii* (*Azotobacter agile* var. *vinelandii*). One of the difficulties foreseen was that the reagents used for preparation of the protoplasts, especially the chelating agents such as Versene (sodium ethylenedinitrilotetraacetate), might inhibit fixation through binding of ions essential for fixation (calcium, iron, molybdenum). In an attempt to circumvent such inhibition two variations in the technique were introduced: (1) the substitution of other metal binding agents; (2) use of penicillin.

Methods.—Culture of Microorganisms: *Azotobacter vinelandii* strain O was grown as described by Esposito and Wilson.[2] Cells intended for protoplast preparations were incubated at 30°C until they had reached the early logarithmic phase of growth, usually 14–16 hr. Such a culture had reached a turbidity of 125–145 Klett-Sumerson units (600 mμ) at this age. Suspensions diluted in Burk's medium, 2 per cent sucrose, were counted in a Spencer Bright Line Improved Neubauer counting chamber as described by Wilson and Knight.[3] Viable counts were made after 48 hr with the aid of a Quebec counter. All suspensions were diluted in Burk's medium, 2 per cent sucrose. Spread plates were made on the same medium and, at times, on agar containing 0.5 per cent sucrose and 0.5 per cent yeast extract.

Preparation of Protoplasts: Protoplasts were prepared by procedures based on the techniques described by Lederberg,[4] Repaske,[5] and Rotman.[6] Since most of the research reported in this paper is based on the protoplasts made by variations in the method of Repaske, details of only these will be supplied. Cells were in the early logarithmic phase of growth when harvested. The incubation mixture consisted of: 2 ml of tris (hydroxymethyl) aminomethane buffer containing sucrose, 1 ml of sodium (diethylenedinitrilo) tetraacetate (Versene), 2 ml of lysozyme, water washed cells and deionized water to a total volume of 6 ml. Such mixtures had an initial turbidity of 300–310 Klett-Sumerson units and a pH of 7.8. The final concentrations per milliliter were: 33 μM tris, 1.4 μM Versene, 13.3 μg lysozyme, 200–250 μg cell nitrogen, 0.06 mM sucrose. Under these conditions, the rods changed to osmotically sensitive spheres in 15 to 30 minutes at room temperature. Five-tenths ml of 1.2 M magnesium sulfate (92 μM/ml) was added when microscopic examination indicated that the reaction was complete. After five minutes further incubation, the protoplasts were centrifuged at 400 × G and washed in sucrose or lactose solutions buffered at pH 7. For fixation experiments, protoplasts were resuspended in Burk's nitrogen-free medium in 0.06 M sucrose. Lysozyme and versene were omitted consecutively from the incubation mixture to establish the effect of these agents. In some experiments potassium citrate replaced versene.

Experimental Results.—Hydrogenase: Table 1 lists the distribution of hydrogenase in whole cells, protoplasts, protoplast membranes, and soluble protoplast fractions. When protoplast membranes are broken with sonic oscillation, most of the activity is found in the fraction sedimenting after 4 hr at 29,000 × G. Apparently,

TABLE 1

DISTRIBUTION OF HYDROGENASE IN *Azotobacter vinelandii* O

Fraction	$Q_{H_2}(N)$*
Cells	8,450
Protoplasts	3,280
Lysed protoplasts	1,300, 1,765
Protoplast membrane	600
Protoplast soluble fraction	0

* $Q_{H_2}(N)$ = μL H_2 taken up/hr/mg N. Hydrogenase activity was determined manometrically using an atmosphere of 100% H_2. Each Warburg vessel held: (*a*) 300 μM of potassium phosphate, pH 8; (*b*) 25 μM methylene blue chloride; (*c*) about 50 μg cell nitrogen, and (*d*) water for a total of 3.2 ml. In all manometric trials (hydrogenase and respiration) the protoplasts were stabilized in 5 per cent lactose–2 μM $MgSO_4$.

hydrogenase in the azotobacter is a particulate system localized exclusively in the membrane.[7]

Oxidative Properties: Typical results of experiments in which the ability of the protoplasts prepared by the lysozyme-Versene technique to oxidize various substrates are shown in Figures 1 and 2. In general the observed rate was not as high as that of intact cells; lysis of the protoplasts lowers the activity further. Versene, citrate, and Amberlite M-B-3 resin have been used as the metal binding agents, but the most satisfactory results were obtained with the lysozyme-Versene preparations. With citrate as a metal binding agent, high endogenous oxidation may result in protoplast preparations; hence, this method is not satisfactory for respiration experiments. The cause of the high endogenous value is undoubtedly oxidation of the citrate reagent which depends upon the magnesium to citrate ratio.[8]

Nitrogen Fixation: Table 2 summarizes the results from numerous experiments on uptake of N_2^{15} by protoplasts of *Azotobacter vinelandii* O. Early in the investigations, 4 hr exposure to N_2^{15} was used, but as this time accentuates the effect of

TABLE 2

N_2^{15} UPTAKE IN ATOM PER CENT EXCESS BY PROTOPLASTS OF *Azotobacter vinelandii* O

A. Lysozyme sensitivity induced by versene

0.050	0.020	0.003	0.446	0.105	0.009
0.000	0.002	0.016	0.464	0.147	0.294
0.031	0.018	0.091	0.089	0.247	0.046
0.037	0.073	0.015	0.020	0.345	0.042
0.013	0.016	0.018	0.092	0.515	
0.032	0.006	0.010			

B. Lysozyme sensitivity induced by Amberlite M-B-3

Protoplasts	0.08, 0.001, 0.030, 0.011
Deionized cells	0.357, 0.028
Cells plus lysozyme only	0.124, 0.142

C. Lysozyme sensitivity induced by citrate

Protoplasts	0.033, 0.014
Citrate treated cells	0.064, 0.048
Whole cell control	0.293

D. Control Preparations

10^5 cells per ml (0.3 μg cell nitrogen per ml)	
Plus:	
180 μg serum albumin per ml	0.011, 0.006
500 μg serum albumin per ml	0.003, 0.000
washed protoplast suspension	0.020
Lysed protoplast preparation containing maximum of 10^5 viable cells per ml	0.000, 0.000

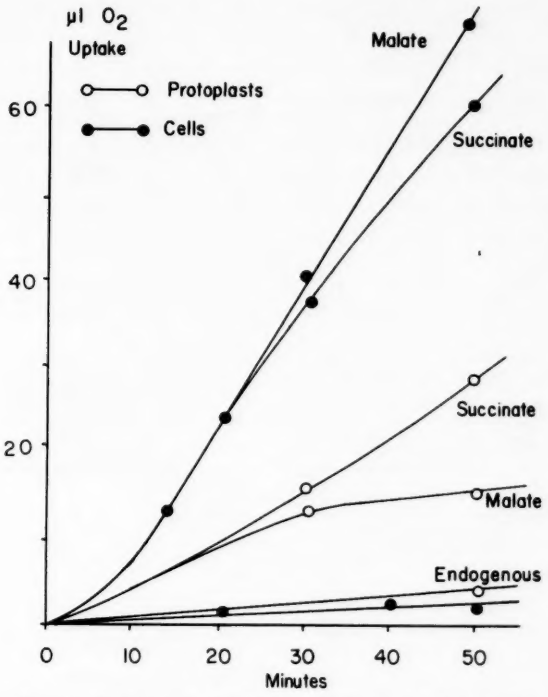

FIG. 1.—Comparison of oxidation of succinate and malate by whole cells and protoplasts of *Azotobacter vinelandii* O. Oxygen uptake was measured in an atmosphere of 50-50 mixture of helium and oxygen by standard manometric techniques. Each flask held: (*a*) 80 µM potassium phosphate pH 7; (*b*) 2 µM magnesium sulfate; (*c*) suspension equivalent to 0.1 mg of cell nitrogen; (*d*) 0.2 ml of 20 per cent KOH in the center well; (*e*) and 40 µM substrate in the side arm; (*f*) total volume, 3.2 ml. With succinate and malate, 1–2 µM of acetate "sparker" was included. To stabilize the protoplasts, lactose to a final concentration of 2.5 per cent was added with magnesium sulfate.

viable cells present, it was shortened to 1–2 hr. The level of whole cells in protoplast preparations is indicated by the viable count.

Although incorporation of N_2^{15} by protoplast preparations is definite, fixation is not as great as with intact cells. The reasons may be the presence of ammonia, action of versene, or high protein content (from lysed protoplasts). Ammonia rapidly and extensively inhibits nitrogen fixation;[9] however, quantitative analysis of a lysed protoplast preparation by the Conway diffusion technique showed ammonia to be absent. The data in Table 3 suggest that versene is responsible for at least part of the impaired fixation of protoplasts. Versene reduces viability of water washed cells below 1 per cent. The function of antibiotics as chelating agents has been discussed by Weinberg,[10] who reported Fe^{++} reversed inhibition of *Azotobacter vinelandii* by tetracycline. In our experiments, Fe^{++} (as Fe^{++} citrate complex) and ammonia enabled growth (measured turbidimetrically) of versene-treated

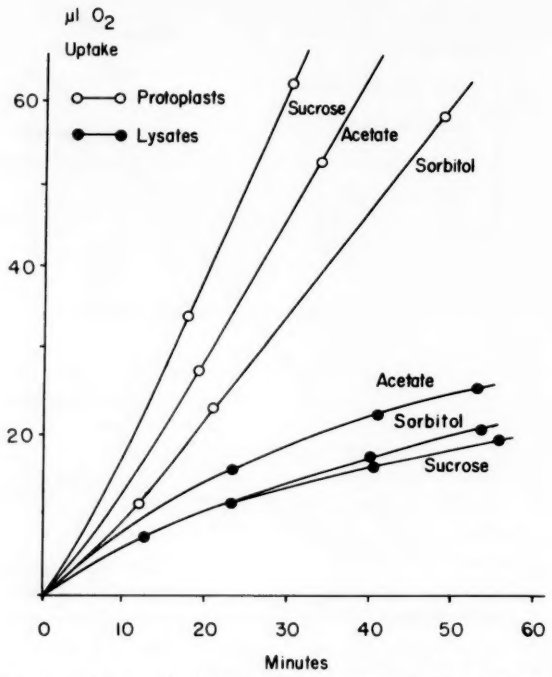

FIG. 2.—Effect of lysis on oxidative properties of protoplasts of *Azotobacter vinelandii* O. Same conditions as in Fig. 1.

cells to resume for about 4 hr. Versene-treated cells assimilated N_2^{15} comparable with that by protoplasts. Cells with versene omitted in the treatment mixture (i.e., exposed only to enzyme) were unaffected in N_2^{15} uptake.

Because ferrous ion partially reversed versene inhibition of cells, its action on N_2^{15} uptake was observed. On both versene-treated cells and protoplasts, Fe^{++} is inhibitory at a level of 50 μg per ml. Atom per cent excess values of 0.01, 0.013 (treated cells) and 0.45, 0.092 (protoplasts) were obtained. A protoplast control showed 0.400 atom per cent excess. The growth constant of whole cells, however, is little changed by 50 μg per ml Fe (as citrate complex).

The high protein content owing to lysed protoplasts is probably not inhibitory to fixation. Nason, Takahashi, Hoch, and Burris[11] state that the 15–40 per cent whole

TABLE 3

THE EFFECT OF REAGENTS USED TO PRODUCE THE PROTOPLAST ON N_2^{15} UPTAKE

	Fraction	μg N/ml	Total Count	Viable Count	Atom % Excess
I.	Protoplasts	418	10×10^8	9×10^4	0.446, 0.464
	Cells, versene treated	405	6×10^8	6×10^6	0.400
	Cells, lysozyme treated	400	...	...	0.530, 0.680
II.	Cells	208	16×10^8	5×10^8	0.949
	Cells, lysozyme treated	200	8×10^8	4×10^8	0.950

Protoplasts were prepared by use of the versene-lysozyme method. In the experiments reported in Tables 2 and 3, uptake of N_2^{15} was determined by the conventional methods on suspensions that were incubated under a gas mixture of: 0.1–0.2 atom N_2 (32 atom per cent N_2^{15}), 0.4 atom O_2, and 0.5–0.4 atom He.

cells remaining in a sonically disintegrated preparation fix with twice the efficiency of comparable whole cell controls.

The crucial question, of course, is not the magnitude of the observed fixation, but is it real or only an experimental artifact arising from the whole cells remaining in the preparation. Routine counting established that intact cells ranged between 10^4–10^5/ml. Controls using 10^5 cells/ml took up no detectable N_2^{15} during a 2-hr exposure. It is recognized, however, that such controls do not accurately reproduce the conditions in the protoplasts preparations. In an effort to simulate these conditions, serum albumin was added to such controls (to correspond to protein in the protoplasts) as well as lysed protoplasts. As is evident from the data in Table 2D, none of these additions induced whole cells at a concentration of 10^5/ml to take up appreciable quantities of N_2^{15} during a 2-hr exposure.

* This research was supported in part from grants-in-aid from the National Science Foundation (G-2826) and from the National Institutes of Health, U. S. Public Health Service (E-1417, C4).

† Subcellular spherical particles prepared as described in this report have been termed "protoplasts" by most investigators. For the reasons cited by a group of workers in this field in a recent note (*Nature*, **181**, 1713–1714, 1958) this may be a misnomer—such particles often are not analogous to the protoplasts (as originally defined by Weibull) in that the entire cell wall may not be removed by the treatment. No simple replacing term, however, was proposed; one might note this limitation by placing quotation marks around the term, but this device becomes awkward when constantly repeated. This footnote acknowledges that this qualification is understood and accepted even though the term is retained in the text.

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DIHYDROTHIOCTYL DEHYDROGENASE—A FLAVOPROTEIN

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The α-ketoglutaric (KG) dehydrogenase purified from hog heart mitochondrial preparations is a multifunctional enzyme complex of molecular weight 2×10^6 and diameter 120 Å. It contains 8 to 10 per cent lipide, and two tightly bound nondialyzable cofactors, diphosphothiamine (DPT) and thioctic (lipoic) acid.[1] Two other coenzymes, diphosphopyridine nucleotide (DPN) and coenzyme A (CoASH), participate in the physiological oxidation as represented in equation

(1).[2, 3] There is considerable evidence[4, 5] that this reaction proceeds in at least four steps as shown in equations (2) to (5). The scheme is similar to that proposed

$$\text{R—CO—COOH} + \text{DPN}^+ + \text{CoASH} \rightarrow \text{R—CO—S—CoA} + \text{DPNH} + \text{H}^+ + \text{CO}_2. \quad (1)$$

$$\text{R—CO—COOH} \rightleftarrows [\text{R—CHO}] + \text{CO}_2 \quad (2)$$

$$[\text{R—CHO}] + \left[\text{T}\begin{matrix}\text{S}\\|\\\text{S}\end{matrix}\right] \rightarrow \left[\text{T}\begin{matrix}\text{S—CO—R}\\\diagdown\\\text{SH}\end{matrix}\right] \quad (3)$$

$$\left[\text{T}\begin{matrix}\text{S—CO—R}\\\diagdown\\\text{SH}\end{matrix}\right] + \text{CoASH} \rightarrow \left[\text{T}\begin{matrix}\text{SH}\\\diagdown\\\text{SH}\end{matrix}\right] + \text{R—CO—S—CoA} \quad (4)$$

$$\left[\text{T}\begin{matrix}\text{SH}\\\diagdown\\\text{SH}\end{matrix}\right] + \text{DPN}^+ \rightarrow \left[\text{T}\begin{matrix}\text{S}\\|\\\text{S}\end{matrix}\right] + \text{DPNH} + \text{H}^+. \quad (5)$$

for pyruvate oxidation.[6] The intermediates shown in brackets in the equations do not appear to dissociate from the enzyme complex. In the present communication, evidence for the involvement of another coenzyme, flavin adenine dinucleotide (FAD), in KG oxidation (specifically, in the dihydrothioctyl dehydrogenase step) is presented.

The highly purified KG dehydrogenase shows the following enzymatic activities besides catalyzing the physiological reduction of DPN and formation of succinyl CoA: (*a*) decarboxylation of KG to succinic semialdehyde[1] and exchange of $C^{14}O_2$ with the α-carboxyl[7] [equation (2)]; (*b*) formation of succinohydroxamate without the participation of CoA, which is indicative of a succinyl-enzyme intermediate[5] [equations (2) plus (3)]; (*c*) reduction of 2,6-dichlorophenol indophenol (DCIP) or ferricyanide by KG;[1] (*d*) reversible reduction of thioctamide by DPNH[4] [equation (5)]; (*e*) reduction of DCIP by DPNH[4] [equation (6) or diaphorase activity related to equation (5)]. The requirement of CoASH for the synthesis of arsenite-inhibited enzyme provides indirect evidence for equation (4).[5]

$$\text{DPNH} + \text{H}^+ + \text{Dye} \rightarrow \text{DPN} + \text{Dye H}_2. \quad (6)$$

In an attempt to separate the subunits of function and obtain direct evidence for the intermediate steps, the KG dehydrogenase was digested with trypsin (1.0 mg trypsin per 100 mg dehydrogenase at 20°C) which resulted in loss of the physiological activity within 5 min. The DCIP reduction (by KG) activity dropped equally rapidly to one-half of the initial activity followed by a much slower gradual decrease. The thioctic reduction and diaphorase activities, on the other hand, increased to roughly 150 per cent of the initial activity, remained constant for at least 60 min, and then declined gradually. The untreated enzyme sediments as a pellet when centrifuged at 144,000 $\times$ *g* for 45 min. Under similar conditions, roughly 80 per cent of the digested protein remained in the supernatant. The above-mentioned activities were present in the supernatant fraction, the small pellet being completely inactive. These results indicate the disruption of the integrated enzyme complex by trypsin into smaller fragments and simultaneous destruction of one (or more) of the intermediate steps in the sequence. The overall activity was not restored by the addition of DPT and Mg^{++}.

Fractionation of the KG dehydrogenase after mild trypsin digestion was successful in separating the activities. The precipitate obtained at 0.30 to 0.40 saturation of ammonium sulfate catalyzed the oxidation of KG by DCIP, and the fraction precipitating between 0.50 and 0.60 saturation contained the $T(SH)_2$ dehydrogenase (and diaphorase) with no cross-contamination of activities.

The $T(SH)_2$ dehydrogenase has been purified to a stage where it appears as a single zone after electrophoresis on paper for 16 hr at pH 7.2 and 8.3. During the purification the ratio of dehydrogenase to diaphorase activity remains constant. The following evidence shows that enzyme is a flavoprotein with FAD as the prosthetic group: (1) The absorption spectrum in the visible region of the protein-free extract obtained by heat or acid denaturation of the protein was similar to the spectrum of FAD (maxima at 370 mμ and 450 mμ). The average FAD content of the preparation was 0.92 per cent or 1 mole in 86,000 g. (2) Paper chromatography of the extract in butanol-acetic acid-water[8] and 5 per cent disodium phosphate[9] gave single spots with R_f values identical with the R_f of authentic FAD. Mixed chromatography failed to resolve the two compounds while flavin mononucleotide was readily separated from the prosthetic group. (3) The flavin from the dehydrogenase restored the activity of apo-D-aminoacid oxidase. The K_m was 1.84×10^{-7} M compared to the value of 1.97×10^{-7} M for authentic FAD. (4) In common with most flavoproteins, this enzyme has absorption peaks at 360–365 mμ and 456 mμ with a shoulder at 485 mμ (Fig. 1, curve 1). The enzyme is almost completely bleached by excess hydrosulfite (curve 2) and only partially

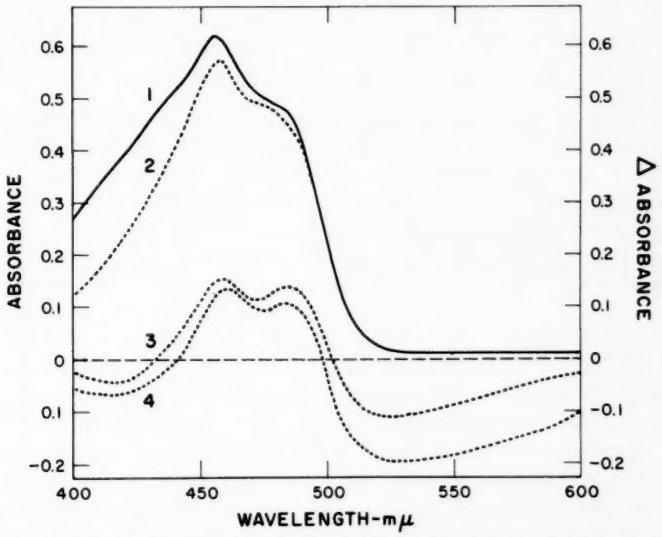

FIG. 1.—Spectral properties of dihydrothioctyl dehydrogenase. Curve 1—Enzyme (5.4 mg. containing 0.061 μ mole FAD in 1.0 ml.) (curves 2 to 4 were obtained by subtracting the spectrum of reduced enzyme from curve 1). Curve 2—Difference spectrum after reduction with excess $Na_2S_2O_4$. Curve 3—Difference spectrum after reduction with 1.2 μ moles of (−) dithiolactanoate. Curve 4—Difference spectrum after reduction with 1.5 μ moles of DPNH.

by dithioloctanoate and DPNH (curves 3 and 4, respectively) under essentially anaerobic conditions. From the extent of reduction at seven different DPN:DPNH ratios, the E'_0 has been calculated to be in the region of -0.34 volts at pH 7.0 and 22° C. The appearance of absorption in the region between 500–600 mμ on reduction (indicated by valley in the difference spectra—curves 3 and 4) is similar to the observations of Beinert[10] which have been attributed to the formation of a relatively stable semi-quinone intermediate. The peaks near 420 mμ produced by enzymatic reduction and not seen in hydrosulfite reduced enzyme are of unknown significance.

After these experiments were completed, a report by Reed[11] appeared indicating that the pyruvic dehydrogenase complex from *E. coli* is partially reactivated by FAD after treatment with acidic ammonium sulfate. He concludes that the FAD is "presumably associated with dihydrolipoic dehydrogenase." Massey[12] has observed recently that a preparation of diaphorase obtained by mild treatment has thioctic reduction activity. We have confirmed that diaphorase prepared essentially by the method of Straub[13] has reductase activity. It is also significant that both the dehydrogenase and diaphorase have high fluorescence. These results may provide the answer to the long-standing question of the physiological role of diaphorase.

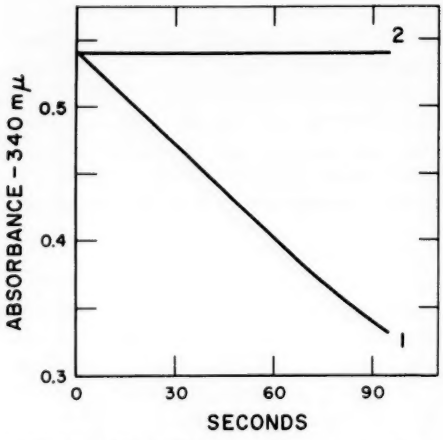

FIG. 2.—Dihydrothioctyl dehydrogenase from chloroplast extracts. Curve 1—Complete system including 1×10^{-4} *M* DPNH, 7.5×10^{-4} *M* DL-thioctamide and 0.26 mg protein in 1 ml of 0.05 *M* phosphate at pH 6.8. Curve 2—No thioctamide.

A similar dihydrothioctyl dehydrogenase activity is present in extracts of oncewashed chloroplast preparations (kindly provided by Dr. A. Krall) from spinach. The extract was made by stirring the chloroplast suspension in 5 per cent chloroform for 30 min followed by centrifugation and dialysis. The extract was able to catalyze the oxidation of 6 μ moles of DPNH per hr per mg protein (or 14 μ moles of DPNH per hr per mg on the basis of the chlorophyll before extraction) (Fig. 2). Under these assay conditions TPNH was completely inactive. Since the optimum conditions for efficient extraction and for stability of the enzymatic activity have not been standardized, it is difficult to relate the extracted activity to the activity of the enzyme in the intact chloroplast. The presence of the flavoprotein in a photosynthetic system may be of significance in view of the postulated role of thioctic acid in the Hill reaction.[14]

Summary.—The α-ketoglutaric dehydrogenase complex has been separated into two fractions. One of them catalyzes the reduction of dyes by α-ketoglutarate. The other fraction which has thioctic reductase and diaphorase activities is a

flavoprotein of high reducing potential (E'_0 approximately -0.34 v.). Thioctic reductase is present also in spinach chloroplasts.

Note added in proof.—Since this paper was submitted for publication, Massey [*Biochim. Biophys. Acta*, **32**, 286, (1959)] has provided additional evidence that diaphorase is a component of the KG dehydrogenase complex.

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THE ATP-DEPENDENT REDUCTION OF SULFATE WITH HYDROGEN IN EXTRACTS OF DESULFOVIBRIO DESULFURICANS

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Enzymatic systems that reduce sulfate seem to be widespread in nature since many organisms can satisfy their nutritional requirements for sulfur with sulfate. The ability to reduce sulfate is most highly developed in certain microorganisms, e.g., *Desulfovibrio desulfuricans*, in which inorganic sulfate functions in anaerobic respiration as the terminal electron acceptor.[1] *D. desulfuricans* can grow heterotrophically with sulfate and an organic electron donor or autotrophically with sulfate, hydrogen, and carbon dioxide.[2] Reaction (1) illustrates the reduction of sulfate to sulfide with molecular hydrogen.

$$SO_4^{--} + 4H_2 \rightarrow S^{--} + 4H_2O \qquad (1)$$

The change in free energy is sufficient for formation of at least four high-energy bonds. It is assumed that, at some point in the enzymatic sequence catalyzing reaction (1), the energy required for autotrophic growth is generated.

Although whole cells of *D. desulfuricans* utilize hydrogen in the presence of sulfate, the inability of cell-free extracts to reduce sulfate has been observed repeatedly by Postgate[1] and Ishimoto *et al.*[3] Sulfite, thiosulfate, and tetrathionate are reduced to hydrogen sulfide by cell-free extracts.[3] S^{35}-labeled sulfite has been isolated from reaction mixtures in which whole cells of *D. desulfuricans* reduced sulfate with hydrogen.[4] This observation, together with other data,[5] suggested that only free sulfite or a compound on the oxidation level of sulfite is an intermediate

in sulfate reduction by this organism. Whether the intermediates are free-sulfur compounds or derivatives of them, as has been postulated for nitrate reduction,[6] has not been determined.

D. desulfuricans is also unique among the nonphotosynthetic anaerobes in that the organism contains large amounts of a cytochrome identified as cytochrome c_3.[7] This cytochrome ($E_0' = -0.205$) functions as an electron carrier in the production of hydrogen from formate and couples sulfite and thiosulfate reductases with hydrogenase. It is also implicated in sulfate reduction, since reduced intracellular cytochrome c_3 is oxidized when sulfate is added to a suspension of whole cells.[1, 8]

In this communication some properties of a cell-free system that reduces inorganic sulfate with hydrogen will be described.

Materials and Methods.—*Desulfovibrio desulfuricans* was grown as described by Peck and Gest,[9] except that the alkaline pyrogallol seal was omitted and the 12-liter flasks were sealed with a sterile rubber stopper. Cell-free extracts were prepared by passing 30–40 g wet weight of cells suspended in 100 ml of 0.05 *M* tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.75, once through a French pressure cell (American Instrument Corporation). The extract was centrifuged at 30,000 × *g* for 30 minutes and then stored under hydrogen in an ice bath or at −20°C. Extracts prepared in this manner contained 50–60 mg of protein per milliliter.

Other preparations were made up as follows: (*a*) Cytochrome c_3—crude extract passed through Amberlite IRC-50 column[8] prepared as described by Margoliash;[10] column washed with distilled water and 0.02 *M* phosphate buffer, pH 6.0; cytochrome eluted with 0.25 *M* phosphate buffer (pH 6.0), boiled for 5 minutes, dialyzed overnight at 2°C against distilled water, and lyophilized. (*b*) Adenosine-5′-phosphosulfate—synthesized by method of Baddiley *et al.*[11] (*c*) Inorganic pyrophosphatase by method of Heppel.[12] ATP was purchased from Pabst Laboratories and methyl viologen from British Drug Houses, Ltd., Toronto. Sulfurylase (Fraction VII) was a gift of Dr. Phillips Robbins.

Determinations were made as follows: Cytochrome c_3 concentration, spectrophotometrically with a 5.4×10^4 molar extinction coefficient;[1] hydrogen utilization, in conventional Warburg apparatus at 30°C; inorganic phosphorus, by method of Fiske and SubbaRow;[13] pyrophosphate, by method of Flynn *et al.*;[14] sulfite, titrated with sodium thiosulfate;[15] protein, by method of Bucher.[16]

Results.—The reduction of sulfate by intact cells of *D. desulfuricans* is inhibited by selenate.[17] Ishimoto *et al.*[3] demonstrated that tungstate also will inhibit sulfate reduction. In both instances, however, the effect of these anions on sulfite reduction was small. Robbins and Lipmann[18] showed that the first step in the activation of sulfate by extracts of baker's yeast is the formation of adenosine-5′-phosphosulfate (APS) and pyrophosphate (PP) from ATP and sulfate as shown in reaction (2).

$$\text{ATP} + \text{SO}_4^{--} \rightarrow \text{APS} + \text{PP} \qquad (2)$$

Wilson and Bandurski[19] observed that the enzyme sulfurylase catalyzes a rapid liberation of inorganic phosphate in the presence of inorganic pyrophosphatase and Group VI anions (MoO_4^{--}, SeO_4^{--}, WO_4^{--}, and CrO_4^{--}). The selenate

and tungstate inhibition of sulfate reduction by whole cells of *D. desulfuricans* suggested that the first step in sulfate reduction might be the ATP-dependent activation of sulfate shown in reaction (2).

The effect of molybdate, tungstate, and chromate on phosphate liberation from ATP by a cell-free extract is shown in Table 1. Crude cell-free extracts exhibit

TABLE 1

EFFECT OF MOLYBDATE, TUNGSTATE, AND CHROMATE ON THE RELEASE OF INORGANIC PHOSPHATE FROM ATP IN CELL-FREE EXTRACTS

Reaction Mixture*	Activity, μmoles of *Pi*/hr/mg of protein
Complete	0.08
Minus Na_2SO_4	0.24
Minus ATP	0.0
Plus 10 μmoles of molybdate	3.10†
Plus 10 μmoles of tungstate	1.80†
Plus 10 μmoles of chromate	2.94†

* Complete system: 100 μmoles of Tris buffer pH 7.5, 2 μmoles of $MgCl_2$, 5 μmoles of ATP, 10 μmoles of cysteine, 10 μmoles of Na_2SO_4, 0.02 ml of pyrophosphatase. Total volume, 0.5 ml., temp., 30°C.
† Na_2SO_4 omitted.

a strong inorganic pyrophosphatase activity and also catalyze the liberation of inorganic phosphate from ATP when molybdate, tungstate, or chromate is included in the reaction mixture. An increase in the liberation of inorganic phosphate in the presence of sulfate was not observed in crude extracts. Liberation of inorganic phosphate was sulfate dependent after streptomycin precipitation and dialysis to remove endogenous sulfate.

Since these observations suggested that the extract could probably form APS, I attempted with these extracts to observe the utilization of hydrogen for sulfate reduction in the presence of ATP. Table 2 shows that hydrogen was consumed

TABLE 2

EFFECT OF ATP, MOLYBDATE, TUNGSTATE, AND CHROMATE ON SULFATE REDUCTION WITH HYDROGEN IN WHOLE CELLS AND CELL-FREE EXTRACTS

Reaction Mixture	Activity, μl of H_2/15 min	
	Whole Cells*	Cell-Free Extract†
Complete	175	86
Minus Na_2SO_4	0	0
Minus ATP	314	0
Plus 10 μmoles of molybdate	0‡	0
Plus 10 μmoles of tungstate	0‡	0
Plus 10 μmoles of chromate	0‡	0

* Complete system: 125 μmoles of phosphate buffer pH 7.15, 10 μmoles of $MgCl_2$, 40 μmoles of Na_2SO_4, 10 μmoles of ATP, 0.5 ml of a 20 per cent suspension of cells. Total volume, 2.0 ml in center well; 0.2 ml 20 per cent KOH in center well; temp., 30°C; gas phase, hydrogen.
† Complete system: 125 μmoles of phosphate buffer pH 7.75, 10 μmoles of $MgCl_2$, 10 μmoles of ATP, 40 μmoles of Na_2SO_4, 0.8 μmole of methyl viologen, 15 mg of protein. Total volume, 2.0 ml; 0.2 ml 20 per cent KOH in center well; temp., 30°C; gas phase, hydrogen.
‡ ATP omitted.

in the presence of both ATP and sulfate but not in the presence of ATP or sulfate alone. ATP was not required by the whole cells for sulfate reduction and actually seemed to inhibit the reduction. Tungstate, molybdate, and chromate completely inhibited sulfate reduction in whole cells and in cell-free extracts. The inhibition of sulfate reduction by molybdate, tungstate, and chromate and liberation of inorganic phosphate from ATP in the presence of these anions suggest that the

first step in sulfate reduction by molecular hydrogen requires the activation of sulfate by ATP with the formation of APS [reaction (2)].

The enzyme system is stable to freezing and also to storage at 2°C. After centrifugation at 120,000 × g for 1 hour, 50 per cent of the activity is lost. Addition of the particles, which show no sulfate-reducing activity by themselves, to the soluble extract completely restores the original activity. This stimulation seems to be the result of a portion of the factors, which are concerned in electron transport between hydrogenase and sulfate reductase, remaining on the particles.

Reduction of sulfite and thiosulfate[1, 8] by cell-free extracts requires cytochrome c_3. Cytochrome c_3 is also required for evolution of hydrogen from formate. Although reduced cytochrome c_3 is oxidized by sulfate in whole cells, treatment of such cells with detergent to render them permeable to cytochrome c_3 completely destroys this activity. But the treated preparations could still catalyze the oxidation of reduced cytochrome c_3 with sulfite or thiosulfate.[1] When cell-free extracts were passed through a column of Amberlite resin to remove cytochrome c_3,[8] the thiosulfate reductase and formic hydrogenlyase activities were lost. Addition of cytochrome c_3 completely restored both activities. As shown in Table 3, passage of the

TABLE 3

STIMULATION OF SULFATE REDUCTASE BY CYTOCHROME c_3 AND METHYL VIOLOGEN

Extract	Reaction Mixture*	Activity, μl of H_2/hr/mg of protein
Crude	Complete	5.2
Crude	Minus ATP	0.7
Amberlite treated	Complete	0.4
Amberlite treated	Plus 1 mg. of cytochrome c_3	6.0
Amberlite treated	Plus 0.8 μmole of methyl viologen	47.3

* Complete system: 300 μmoles of Tris buffer pH 7.5, 10 μmoles of $MgCl_2$, 40 μmoles of Na_2SO_4, 10 μmoles of ATP. Total volume, 2.0 ml; 0.2 ml of 20 per cent KOH in center well; temp., 30°C; gas phase, hydrogen.

extract over an Amberlite column also completely inactivates the sulfate reductase, but the activity can be restored by addition of purified cytochrome c_3. Addition of methyl viologen stimulates sulfate reduction eightfold over the activity of the crude extract. This indicates that electron transport is the limiting reaction. DPN and TPN could not substitute for methyl viologen in this reaction. Methyl viologen also facilitates the reduction of sulfite and thiosulfate with molecular hydrogen and presumably functions as an efficient electron carrier between hydrogenase and these reductases.[8]

While investigating the pH characteristics of the reaction, I observed something that has proved useful in identifying the products of sulfate reductase. As shown in Figure 1, at pH 7.75 the reaction proceeds rapidly for 15–20 minutes and then quickly stops. At pH 6.0, in the presence of identical amounts of ATP and sulfate, hydrogen consumption continues for 35–40 minutes, and the total hydrogen uptake is twice as great at this pH as at pH 7.75. The product of the reaction at pH 7.75 was tentatively identified as sulfite. It gave reactions characteristic of sulfite.[20] Titration of reaction mixtures with thiosulfate[3] indicated a 1:1 ratio between the amount of hydrogen utilized and the sulfite produced. At pH 6.0, the product of the reaction seems to be hydrogen sulfide. Probably because sulfite reductase is 6 times as active at pH 6.0 as at pH 7.75 hydrogen utilization is greater and

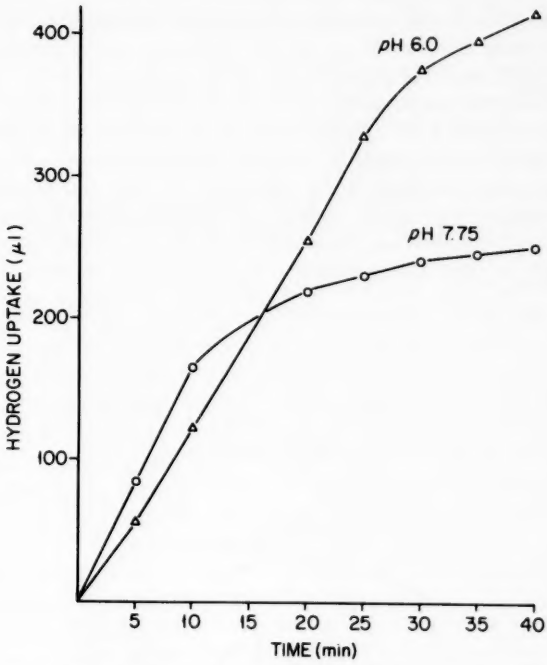

FIG. 1.—Effect of pH on the extent of sulfate reduction. The complete system contained 125 μmoles of phosphate buffer; 10 μmoles of $MgCl_2$; 40 μmoles of Na_2SO_4, 10 μmoles of ATP, 0.8 μmole of methyl viologen, 15 mg of protein. Total volume, 2.0 ml; 0.2 ml 20 per cent KOH in center well; temp., 30°C; gas phase, hydrogen.

hydrogen sulfide is produced. The inhibition of the over-all reduction of sulfate to hydrogen sulfide at pH 7.75 and the low activity of sulfite reductase at this pH suggest that free sulfite, not a bound sulfite, is an intermediate in the reduction of sulfate.

Sulfate activation for sulfate transfer as described by Robbins and Lipmann,[21] and Wilson and Bandurski[22] involves two enzymatic reactions. The first reaction is the formation of APS from ATP and sulfate [equation (2)]. The second is the phosphorylation of APS in the 3′ position to form 3′-phosphoadenosine-5′-phosphosulfate (PAPS) and ADP,[19] shown in equation (3).

$$\text{APS} + \text{ATP} \rightarrow \text{PAPS} + \text{ADP} \qquad (3)$$

In mammalian systems the sulfate of PAPS may be transferred to steroids, phenols and carbohydrates in the presence of the appropriate sulfokinase.[24]

The ATP requirement and the inhibition of sulfate reduction in extracts of *D. desulfuricans* by Group VI anions do not permit us to distinguish between APS and PAPS as the substrate for sulfate reductase. If the intermediate were APS, equal amounts of hydrogen and ATP should be utilized. Also, for each micromole of hydrogen utilized or sulfite produced, 2 μmoles of inorganic phosphate should be

liberated from ATP in the presence of inorganic pyrophosphatase. It should be noted here again that the crude extract contains a potent inorganic pyrophosphatase. If PAPS were the substrate for sulfate reductase and if the presence of myokinase is assumed, a maximum of 0.67 μmole of hydrogen should be consumed per micromole of added ATP. Results of an experiment in which known amounts of ATP were added in the presence of excess sulfate are shown in Figure 2. The amount of hydrogen consumed in the presence of 5, 10, and 15 μmoles of ATP is shown; in each case, 1 μmole of hydrogen is utilized per micromole of added ATP. The curve representing the course of hydrogen utilization with 5 μmoles of ATP should be particularly noted. In this instance, hydrogen consumption completely stopped after 5 μmoles of hydrogen was utilized. This indicates that there was little or no contribution by the product, sulfite, to the total hydrogen uptake. This result suggested that APS rather than PAPS was the substrate for sulfate reductase.

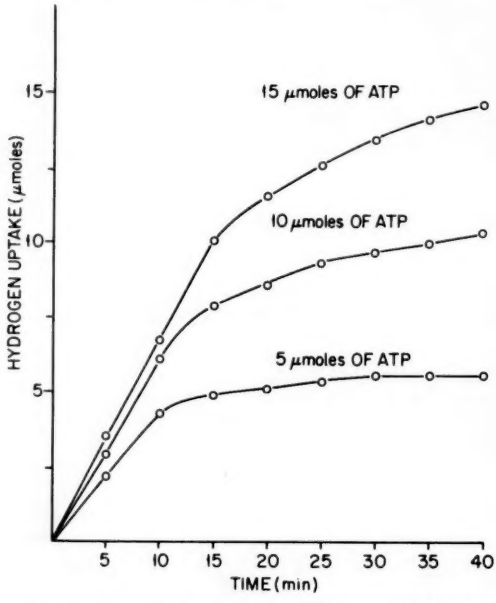

FIG. 2.—The relation between ATP concentration and hydrogen consumption. The complete system contained 125 μmoles of phosphate buffer pH 7.75, 10 μmoles of $MgCl_2$, 40 μmoles of Na_2SO_4, ATP as indicated, 0.8 μmole of methyl viologen, 15 mg of protein. Total volume, 2.0 ml; 0.2 ml 20 per cent KOH in center well; temp., 30°C; gas phase, hydrogen.

A partial balance for the over-all reduction is shown in Table 4. Hydrogen utilization was determined manometrically, sulfite formation by the incorporation of S^{35} in KOH after acidification of the reaction mixture, and ATP utilization by the liberation of inorganic phosphate. The only values subtracted are the zerotime figures. Therefore, 0.4 μmole should be subtracted from the values for hydrogen utilization to correct for the hydrogen uptake caused by the presence of 0.8 μmole of methyl viologen. The values for inorganic phosphate liberation should be regarded as maximum. Electrophoresis of reaction mixtures shows the presence of only AMP and ATP. Thus any 3′,5′-diphosphoadenosines that might have been formed had been hydrolyzed to AMP. The phosphorylation of APS to PAPS and subsequent hydrolysis of 3′,5′-diphosphoadenosine would be expected to increase the ratio of phosphate released to hydrogen utilized, or sulfite produced to a value greater than 2. The observed ratio of 2 is further evidence that APS, not PAPS, is the substrate for sulfate reductase.

When ATP and S^{35}-labeled sulfate were incubated together in crude extracts

TABLE 4

PARTIAL STOICHIOMETRY FOR THE REDUCTION OF SULFATE TO SULFITE WITH HYDROGEN*

Time, min.	μmoles of H_2 Consumed	μmoles of S^{35} Incorporated into KOH	μmoles of *Pi* Liberated
0	0	0	0
10	4.15	3.31	8.1
15	5.9	4.74	9.72
20	7.75	5.45	10.7

* Complete system: 150 μmoles of Tris buffer pH 8.0, 10 μmoles of $MgCl_2$, 12.5 μmoles of $Na_2S^{35}O_4$ (20,000 cpm/μmole), 20 μmoles of ATP, 0.8 μmole of methyl viologen, 15 mg of protein. Total volume, 2.0 ml; 0.2 ml 20 per cent KOH in center well; gas phase, hydrogen; reaction was stopped by the addition of 0.2 ml of 10 *N* H_2SO_4.

under helium or oxygen, $PAPS^{35}$ did not form. However, an S^{35}-labeled nucleotide was formed having the same mobility as APS^{35} prepared by incubation of ATP and S^{35}-labeled sulfate with sulfurylase (Fraction VII) and pyrophosphatase.[16] In enzymatic systems that form PAPS, S^{35}-labeled PAPS can be detected but labeled APS usually cannot. This results from the rapid phosphorylation of APS in the 3′ position to form PAPS.[23] Also, the extract utilizes hydrogen in the presence of crude preparations of APS[11] without additional ATP. Heating under acid conditions, which hydrolyzes the sulfate bond, completely destroys this activity.[24]

Discussion.—An ATP-dependent reduction of S^{35}-labeled sulfate to sulfite[25, 26] and to hydrogen sulfide[26] has been reported to occur in cell-free extracts of baker's yeast. In both cases, PAPS was suggested as the substrate for sulfate reductase.

Evidence that PAPS is not an intermediate in sulfate reduction by extracts of *D. desulfuricans* is presented. However, APS seems to be the first intermediate in sulfate reduction by this system, and the data presented are consistent with the idea that APS is the substrate for sulfate reductase. The product of the reduction, adenosine-5′-phosphosulfite, is unstable and probably immediately decomposes to AMP and free sulfite. This conclusion is based on Wilson and Bandurski's observation[19] that sulfite in the presence of sulfurylase catalyzes the liberation of inorganic phosphate from ATP but does not exchange P^{32}-labeled pyrophosphate into ATP.

The over-all reaction for reduction of sulfate with hydrogen can be represented as follows:

$$H_2 + \text{cytochrome } c_3\ (2Fe^{+++}) \rightarrow \text{reduced cytochrome } c_3\ (2Fe^{++}) + 2H^+$$

$$ATP + SO_4^{--} \rightarrow APS + PP$$

$$APS + \text{reduced cytochrome } c_3\ (2Fe^{++}) + 2H^+ \rightarrow AMP + SO_3^{--} + \text{oxidized cytochrome } c_3\ (2Fe^{+++}) + H_2O \quad (4)$$

The possibility remains, however, that APS itself is not the substrate for sulfate reductase, but rather transfers its sulfate to an unknown sulfate carrier on which the sulfate is then reduced to sulfite.

The extracts used in these experiments were prepared from heterotrophically grown cells. The presence or absence of this system in autotrophically grown cells should be investigated. Assuming for the moment that the system is present in autotrophically grown cells, at least three high-energy phosphate bonds must be generated during reduction of sulfate to hydrogen sulfide since two high-energy bonds are utilized in the activation of sulfate. The free-energy change in the

reduction of cytochrome c_3 with hydrogen is sufficient to permit generation of one high-energy phosphate. Since all the sulfate-reducing enzymes can oxidize cytochrome c_3, their function in anaerobic respiration seems to be analogous to that of cytochrome oxidase in aerobic respiration.

Summary.—A cell-free system from heterotrophically grown cells of *Desulfovibrio desulfuricans* reduces sulfate in the presence of ATP and molecular hydrogen and is completely inhibited by molybdate, tungstate, and chromate. Hydrogenase and the sulfate reductase are coupled by cytochrome c_3 or the dye, methyl viologen. At pH 7.75 the product of the reaction is sulfite, but at pH 6.0 sulfate appears to be reduced to hydrogen sulfide. The stoichiometry of the reaction indicates that 1 μmole of ATP is utilized for each micromole of hydrogen consumed and sulfite produced. These data and the absence of 3′-phosphoadenosine-5′-phosphosulfate formation indicate that adenosine-5′-phosphosulfate is the substrate for sulfate reductase.

I wish to express my appreciation to Dr. G. David Novelli for his advice and encouragement during the course of this investigation.

Note added in proof: Ishimoto has recently reported the ATP-dependent reduction of sulfate to sulfide in cell-free extracts of *D. desulfuricans*. *J. Biochem.* (*Tokyo*), **46,** 105 (1959).

* Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

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*MUTATION INDUCTION AND MACROMOLECULAR SYNTHESIS IN BACTERIA**

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Based on observations that supplementation of the immediate preirradiation growth medium of *Escherichia coli* with RNA purine and pyrimidine precursors leads to an increase in the mutation frequency subsequently induced by ultraviolet light (UV), the hypothesis was advanced that nucleic acid precursors altered *in vivo* by UV are involved as chemical intermediates in the induction of mutation.[1] Witkin[2] has described studies which suggested to her that the mutation induction process is dependent on postirradiation protein synthesis. We confirmed[1] these results with another mutational system and further demonstrated that the increased mutational response to UV produced by preirradiation incubation with nucleic acid precursors was also dependent on postirradiation conditions *favoring* protein synthesis. This information led to the suggestion[1] that UV-induced mutation involves the synthesis of nucleic acid from radiation modified precursors and that this process requires a complex supply of amino acids and possibly protein synthesis.

Later studies[3] of the postirradiation events involved in mutation induction by UV demonstrate that cells held under certain conditions which limit protein synthesis (lack of amino acids or a nitrogen source, chloramphenicol inhibition, etc.) show an immediate decline in mutation frequency which apparently is enzymatically mediated ("mutation frequency decline"). If, however, the cells are held under conditions favoring protein synthesis (in media rich in amino acids) the mutation frequency does not decline. Rather the "potential mutation" is *temporarily* stabilized in the cell ("mutation stabilization"). During this period mutation frequency decline does not occur; however, if conditions unfavorable for protein synthesis are created during this time (i.e., by the addition of chloramphenicol or removal of amino acids, etc.) the mutation frequency will decline. Ultimately the "potential mutation" is "fixed" into the cell ("mutation fixation") and is no longer subject to those conditions which previously resulted in decline in mutation frequency. We thus delineated three basic postirradiation processes which influence the final UV-induced mutation response obtained.

The experiments reported here were designed to elucidate the nature of these postirradiation processes which are involved in mutation induction. An attempt has been made to relate these processes to the macromolecular synthetic activities of *E. coli* following UV exposure.

Materials and Methods.—The mutations studied result in reversion to the non-tryptophan-requiring state of *Escherichia coli* strain WP2 (tryptophan requiring strain of Witkin[2]). In these experiments we chose for our plating medium minimal agar supplemented with 2.5 per cent Difco nutrient broth. The low amino acid concentration in this medium allows a somewhat limited degree of mutation expression and the tryptophan present allows unreverted cells to make enough di-

visions to develop small but visible colonies; therefore, mutations and total survivors can be scored on the same medium. The basic procedures followed for culture growth, synchronization, radiation exposure, culture treatment, and plating have been previously described.[1, 3] UV exposure was 10 sec at 30 cm (964 ergs/mm^2). Unexposed suspensions contained about 6.0×10^8 colony forming cells per ml. UV exposed suspensions contained about 4.5×10^8 colony forming cells per ml. The usual precautions to prevent photoreactivations were observed.

Extraction procedures for nucleic acid and protein were those of Ogur and Rosen.[4] DNA was determined by the method of Burton.[5] DNA was determined by the method of Visser and Chargaff.[6] Protein was determined according to the method of Lowry *et al.*[7]

Photoreversal experiments were carried out by immersing the irradiated cells in small glass tubes in an ice bath six inches from two 500-watt GE projection bulbs. A stream of air was blown over the surface of the apparatus to dissipate heat. Under these conditions maximum photoreversal of mutation was achieved after 30–45 minutes' exposure to light.

Mutation Frequency Decline and Mutation Stabilization.—We have previously reported[3] that in the absence of an adequate nitrogen source or with treatment with chloramphenicol an immediate and rapid decline in UV-induced mutation frequency takes place in the case of the "EMB color" mutations of *E. coli* strain B, and the induced reversion from a tryptophan requirement of *E. coli* strains WP2. For convenience in reference we designated this process "mutation frequency decline" (MFD). We demonstrated that the prevention of MFD depends on the availability of a complex supply of amino acids and suggested that the amino acids are involved in a process of temporary "stabilization" of the "potential mutation" in the cell prior to its primary effect in mutation induction. For convenience in reference and without intending any implication as to mechanism we designated this process "mutation stabilization" (MS).

Figure 1 demonstrates in a comparative manner the time course of MS and MFD. If the cells are plated without incubation onto minimal agar supplemented with 2.5 per cent nutrient broth, the level of mutation which occurs at this particular UV dose yields approximately 4000 prototrophs per 10^8 surviving organisms. The mutation response is limited considerably by the low level of amino acids available in the plating medium. If instead of plating immediately the cells are held in a medium containing an excess of amino acids, a rise in the mutation response will take place with incubation to a maximum level approaching 8000 prototrophs per 10^8 surviving organisms after 40 minutes' incubation prior to plating. This suggests that amino acids have been taken into the cell and actually do something to cause this increased response over that obtained with cells plated immediately on medium with a limited supply of amino acids.

On the other hand, if the cells are held without any amino acids or other nitrogen source, a decline in mutation frequency occurs to a comparatively low basal level. This decline is accomplished in approximately 20 minutes. An almost identical rate of decline is produced in the amino acid supplemented medium with chloramphenicol. Thus chloramphenicol not only prevents the increased response over the 4000 prototroph level obtained upon immediate plating, but promotes the same decline that the complete absence of amino acids promotes. As Table 1 shows, the

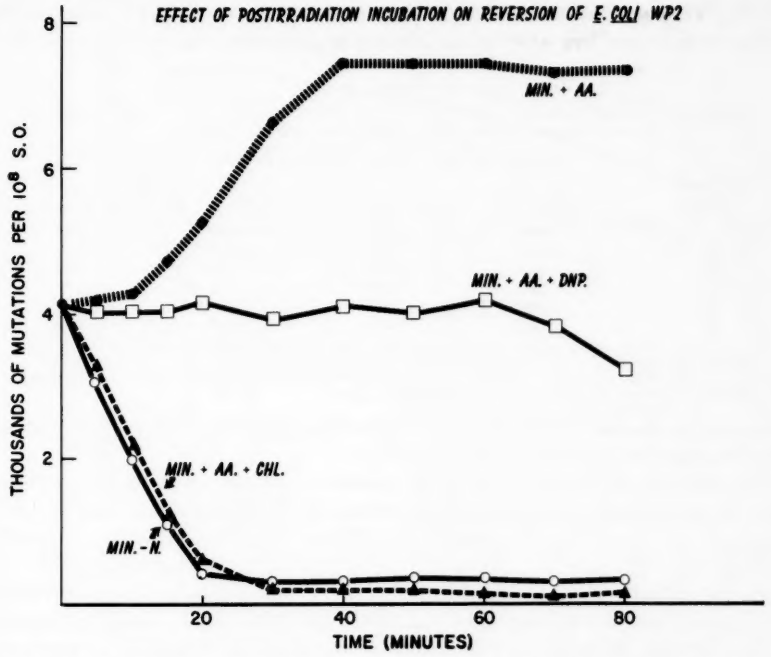

FIG. 1.—A comparison of "mutation stabilization" and "mutation frequency decline" in UV induced reversion of a tryptophan auxotroph, *E. coli* strain WP2, to prototrophy. The UV irradiated cells were held at 37°C on a reciprocal shaker in the following media for the indicated period of time then plated onto minimal[1] agar medium supplemented with 2.5% nutrient broth:[3] (1) Min + AA. = minimal plus casein hydrolysate, NBCo, vitamin free, enzymatic, 2 mg per ml., and *dl*-tryptophan, 0.2 mg per ml; (2) min + AA + DNP = above medium (1) plus dinitrophenol, 5×10^{-3} molar; (3) min + AA + Chl. = above medium (1) plus chloramphenicol, 20 micrograms per ml; (4) min − N = minimal medium with the ammonium sulfate deleted. The plates were incubated 3 days, then scored for mutation and survival.[3] No significant changes in level of survival were produced by the above treatments.

deletion of all amino acids is not required to promote a marked decline in mutation frequency. The elimination of tryptophan alone, the amino acid required by the auxotroph, promotes a similar decline. It therefore appears that not only is the presence of a nitrogen source involved, but a complex supplement of amino acids may be required for the MS process. Evaluation of requirements for other amino acids than tryptophan is complicated by the fact that the organism can synthesize its own supply of the other amino acids. An analysis of the specific amino acids required in the MS process is under way in our laboratory.

In this connection it is interesting to note that Schwartz and Strauss[8] failed to observe the MFD process with *E. coli* strain WP2 in the absence of tryptophan (as described below in Table 1). It seems probable that under the conditions of UV dose, culture growth and manipulation, etc., used by them, their culture does not possess the enzymatic capacity to carry out the MFD process. We have attempted to repeat the results of Schwartz and Strauss in this regard so far without success;

utilizing various conditions of culture growth we have consistently observed the decline in mutation frequency in the absence of tryptophan as reported in Table 1.

TABLE 1

EFFECT OF POSTIRRADIATION INCUBATION ON YIELD OF INDUCED PROTOTROPHS OF *E. coli* STRAIN WP2 (TRYPTOPHAN REQUIRING)

Prototrophs per 10^8 surviving organisms

Incubation Time, Minutes	(1) M - N*	(2) M + AA + T*	(3) M + AA − T*	(4) M + T − AA*
0	4830	4830	4830	2830
10	2280	5020	2520	3500
20	604	5920	1080	2280
30	588	6680	822	1720
40	608	7960	808	1250
50	622	8020	816	858

* Media: (1) M - N = minimal with ammonium sulfate deleted; (2) M + AA + T = minimal plus 20 different amino acids, 0.1 mg per ml each, and DL-tryptophan, 0.2 mg per ml; (3) M + AA − T = media 2 with tryptophan deleted; (4) M + TT − AA = media 2 with the amino acids deleted. Incubation was at 37°C on a reciprocal shaker.

Thus the conclusion of Schwartz and Strauss that MFD requires protein synthesis must be reinterpreted (see a later section).

Our previous studies[3] of MFD in the absence of nitrogen in the medium showed that the decline process has a Q_{10} of approximately 2 between 22° and 37°C. Furthermore, deletion of carbohydrate from nitrogen free medium slowed the decline appreciably. We have also reported[9] previously that dinitrophenol (DNP) prevents the amino acid dependent MS process, and that similarly the elimination of phosphate from the medium prevents MS. In addition the uridine antagonist, hydroxyuridine, prevents the MS process during the first 30 minutes of incubation in the presence of amino acids[9] (see a later section for additional effects of 5-HU). As Figure 1 shows, for over 80 minutes the mutation response in the presence of DNP remains at about the same level upon plating as if the enriched supply of amino acids were not available to the cell. Thus DNP could be interfering with the active absorption of the amino acids or the utilization of the amino acids in the MS process, possibly through its unique capacity to uncouple oxidative phosphorylation. Since mutation frequency does not decline in the presence of DNP (as observed with the *deletion* of amino acids) it was suspected that DNP might also interfere with the alternate process of MFD. Figure 2 demonstrates that this assumption is correct; a decline in mutation frequency comparable to that occurring in nitrogen free medium is not observed when DNP is added to the nitrogen free medium. It appears likely that DNP interferes with that process which acts on the "mutagenic substrate" in the absence of "stabilizing" amino acids. The figure also shows that addition of 5-hydroxyuridine or the elimination of phosphate from the N free medium unlike DNP has little effect on MFD.

The question has arisen as to how real is the mutation frequency decline process, i.e., can it be reversed subsequent to its occurrence by addition of higher levels of amino acids than are available in the plating medium. So far our results have been unqualifiedly negative. Table 2 demonstrates that once MFD has proceeded to any level, the addition of amino acids to the medium does not increase the mutation frequency. The mutation frequency becomes stabilized at the level to which it has declined at the time of addition of amino acids.

The results taken together lend further support to the idea that the ultimate mutation response to a given dose of UV depends on (1) the intracellular supply of

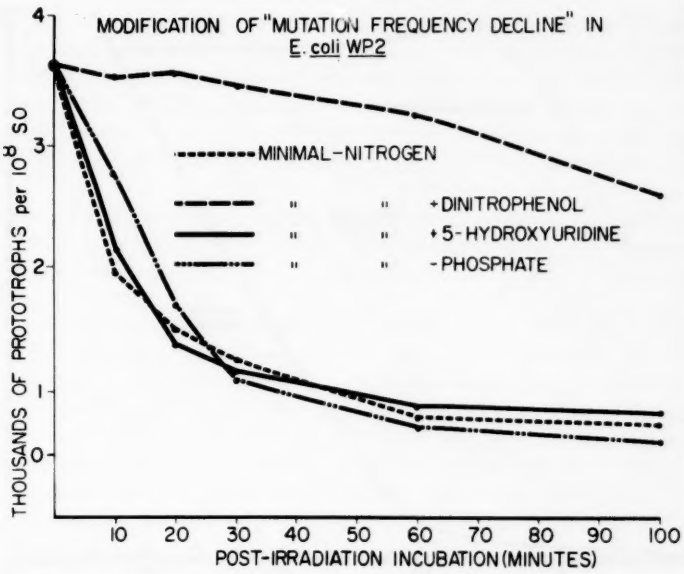

FIG. 2.—Modification of "mutation frequency decline" of UV irradiated *E. coli* strain WP2 held in minimal medium with the ammonium sulfate deleted (minimal − nitrogen). Dinitrophenol was added to a concentration of 5×10^{-3} molar; 5-hydroxyuridine to a concentration of 5 mg per 100 ml. Sodium and potassium phosphate were deleted (− phosphate) and the pH adjusted to 6.8 with hydrochloric acid and potassium hydroxide. No significant changes in level of survival were noted during the course of this experiment.

nucleic acid precursors at the time of UV exposure[1] and (2) the relative efficiency under various conditions following UV exposure of two competitive processes for these precursors: one being amino acid dependent and leading to "stabilization" of the mutagenic nucleic acid precursor (MS) and the other being amino acid independent and leading to the removal of the mutagenic substrate from the pathway of mutation induction (MFD).[3]

Figure 3 demonstrates the postirradiation incubation time relation between MS and subsequent "mutation fixation" (MF) whereby the mutation is irreversibly

TABLE 2

EFFECT OF POSTIRRADIATION SUPPLEMENTATION OF AMINO ACIDS ON MUTATION FREQUENCY DECLINE FOLLOWING UV EXPOSURE OF *E. coli* STRAIN WP2

Time, Minutes, Amino Acids* Added	Yield of prototrophs per 10^8 surviving organisms with incubation at 37°C, minutes										
	0	5	10	15	20	30	40	50	60	70	80
Not added	42	36	22	12	7	5	6	8	7	8	6
0	42	44	50	58	65	75	74	70	71	70	68
5	42	36	36	37	34	32	31	33	35	36	32
10	42	36	22	24	24	26	26	25	26	20	18
15	42	36	22	12	13	12	12	13	13	14	14
20	42	36	22	12	7	7	6	7	6	7	7
30	42	36	22	12	7	5	6	7	6	8	5

* The culture was originally suspended in minimal medium with the ammonium sulfate deleted. At the indicated times amino acids were added to samples and the incubation continued. The amino acids consisted of casein hydrolysate, enzymatic, NBCo, added to a concentration of 2 mg per ml and DL-tryptophan, 0.2 mg per ml,

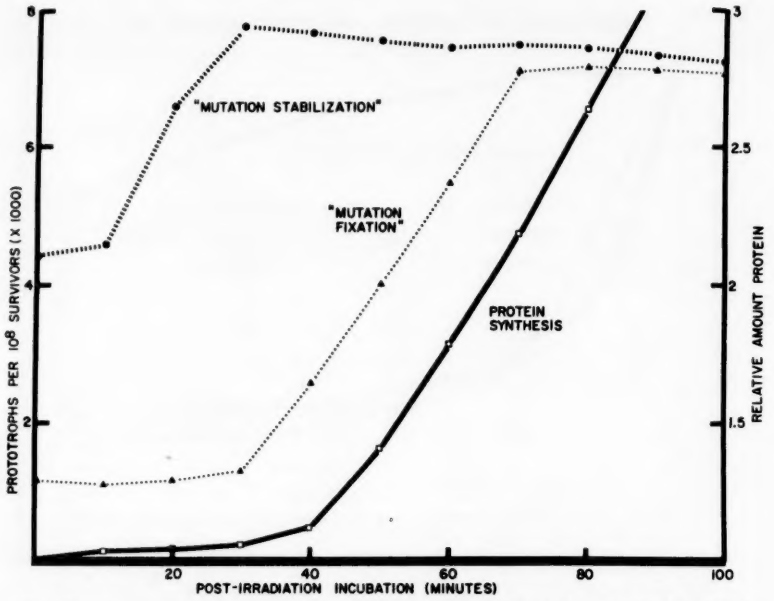

FIG. 3.—A comparison of "mutation stabilization" and "mutation fixation" in UV induced reversion of a tryptophan auxotroph, *E. coli* strain WP2, to prototrophy. The cells were held following UV exposure in minimal medium plus casein hydrolysate, 2 mg per ml., and *dl*-tryptophan, 0.2 mg per ml., on a reciprocal shaker at 37°C and plated at the indicated times ("mutation stabilization"). After these intervals of incubation chloramphenicol was added to samples to a final concentration of 20 micrograms per ml and the samples incubated an additional 45 minutes. This procedure is designated "chloramphenicol challenge" and measures "mutation fixation" since all mutations which remain subject to the chloramphenicol promoted decline process at the time of chloramphenicol addition are eliminated during the subsequent incubation period.[3] Relative protein content of the culture at the times of chloramphenicol addition is also shown (protein synthesis).

fixed into the cell and is no longer subject to conditions which promote MFD. We have previously described[3] the process of MF in the case of the "EMB color" mutations of *E. coli* strain B. The mutation is considered "fixed" if it is no longer subject to the "reversing" effect of chloramphenicol, nitrogen deprivation photoreversal, or other agents or conditions promoting MFD.[3] The technique we have used most frequently in the demonstration of MF is the addition of chloramphenicol to the culture after a given postirradiation incubation interval. This is followed by an incubation period in the presence of chloramphenicol of sufficient duration to interfere with all potential mutations which remain subject to MFD at the time of chloramphenicol addition. We have designated[3] this technique "chloramphenicol challenge." It is clear from Figure 3 that the "potential mutations" are "stabilized" in the cell for a considerable period prior to occurrence of the MF process. During this period they remain subject to agents or conditions promoting MFD. However, after 25 minutes incubation a decreasing number of the potential mutations is influenced by the chloramphenicol and at 75 minutes the mutation frequency response is unaffected by chloramphenicol. At this time we presume all potential mutations are fixed by the occurrence of some synthetic event

in all the cells involved. It is apparent from Figure 3 that neither MS nor MF is correlated with protein synthesis in the culture. Thus the suggestion of Witkin[2] that protein synthesis *per se* is involved in establishment of the level of mutation frequency response appears unacceptable unless one assumes that the protein synthesis so involved is of a quantitatively minor nature (unmeasurable by our methods) and not correlated with the gross protein synthetic activities of the culture.

Mutation Fixation and Photoreversal.—It has been reported that UV-induced mutations may be reversed by white light immediately following UV exposure but that with subsequent incubation these mutations are no longer subject to the reversing effect of light.[10]

Figure 4 shows a comparison between MF as determined by chloramphenicol challenge and photoreversal. The time course of MF measured in these two ways is closely correlated. MF as measured by either is initiated after 15 minutes' incubation and is essentially complete after 75 minutes' incubation. The difference appears to be one of effectiveness, the physical "reversal" by white light being somewhat more efficient than metabolic "reversal" by chloramphenicol. From these results it appears that MF represents a significant and real event in the mutation induction process (see *Discussion*). Further studies of mutation fixation and photoreversal will be described in another publication.

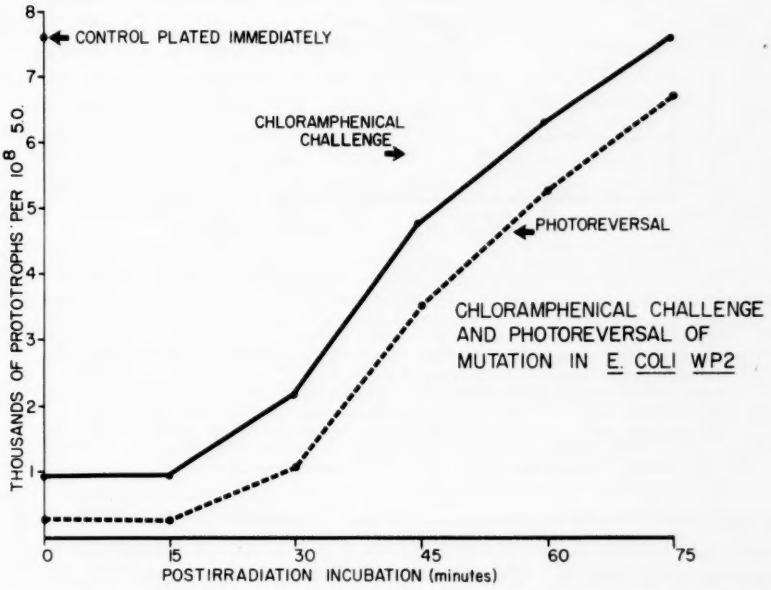

FIG. 4.—A comparison of "mutation fixation" in *E. coli* strain WP2 as measured by "chloramphenicol challenge" and challenge by photoreversal. Following the indicated intervals of incubation after UV exposure aliquots were taken and either incubated 45 minutes more with chloramphenicol prior to plating or exposed to photoreactivating light for a period of 45 minutes. The incubation medium used was minimal plus casein hydrolysate, 2 mg per ml, and *dl*-tryptophan, 0.2 mg per ml. Since little killing is produced by the UV dose used, exposure to light did not modify the survival level significantly.

Mutation Fixation and Macromolecular Synthesis.—We have previously suggested[3] that MF may involve RNA synthesis. This suggestion was based on the following evidence: (1) RNA precursors increase mutation frequency response to ultraviolet light when supplied to *E. coli* prior to UV exposure;[1] (2) cytidine and uridine decrease mutation frequency response when supplied to the culture immediately *following* radiation exposure.[3] On the other hand the addition of thymine or the deoxynucleosides has no effect on the mutation frequency response.[1, 3]

It is obvious that indirect data of this sort can serve only as indicative evidence for the involvement of RNA synthesis in the MF process. We therefore carried out experiments designed to clarify the relation of MF to macromolecular synthetic activities of the culture. Figure 5 relates MF (as determined by "chloramphenicol challenge") to RNA, protein, and DNA synthesis in an irradiated culture of *E. coli*. It is evident that the progression of MF is closely correlated with RNA synthesis in the culture. MF is initiated at the time that RNA synthesis begins and has attained its maximum level at the time that the net amount of RNA has doubled. This particular experiment was chosen for presentation here since for some unknown reason the rate of RNA synthesis shows a decrease during the 35–45 minute period of incubation. A corresponding change in the course of MF is observed. It is interesting that the change in rate observed with RNA synthesis

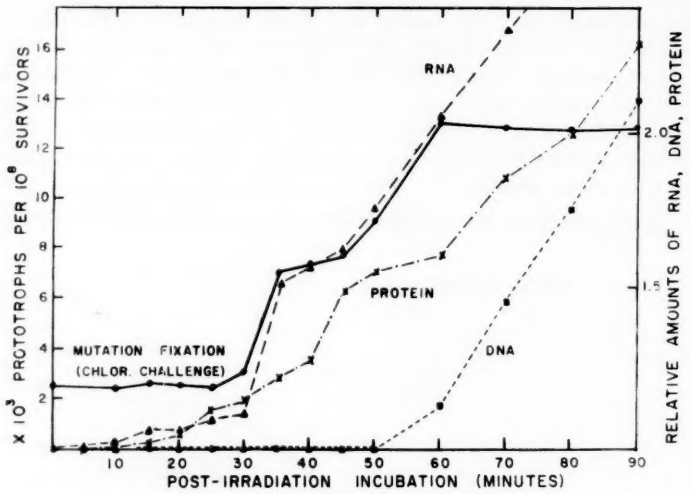

FIG. 5.—The relation of "mutation fixation" as demonstrated by "chloramphenicol challenge" to the synthesis of RNA, DNA, and protein in an UV exposed culture of *E. coli* strain WP2. UV exposure was for 10 sec at 30 cm (964 ergs/mm² below 2,800 A). The unexposed control suspension contained 6.5×10^8 colony forming cells per ml. The exposed culture contained 5.1×10^8 colony forming cells per ml. The bacteria were incubated after radiation exposure at a 1:5 dilution in minimal medium supplemented with 2 mg per ml casein hydrolysate and 0.2 mg per ml *dl*-tryptophan. After the indicated interval of incubation chloramphenicol was added to a final concentration of 20 micrograms per ml and the culture incubated for an additional 45 minutes ("chloramphenicol challenge"). The treatments and incubation did not produce any significant changes in survival. Cell division began after 90 minutes' incubation.

during the period of 35–45 minutes is reflected in a change in rate of protein synthesis from 45 to 60 minutes. This could be taken to suggest a direct sequential relation of RNA synthesis and protein synthesis.

It should be pointed out that this change in rate of RNA synthesis is not our usual finding; rather RNA synthesis proceeds in a linear fashion once initiated. We have retarded the rate of RNA synthesis by addition of 5-hydroxyuridine to the medium during the course of RNA synthesis and MF and, as expected, we find that MF is retarded accordingly. (When 5-hydroxyuridine is added to a culture actively synthesizing RNA, synthesis will be blocked for a short period. The culture then apparently adapts to the presence of 5-hydroxyuridine and synthesis proceeds. The above described results were obtained during that period that 5-HU is effective in blocking RNA synthesis completely.) Both processes resume upon reversal of the 5-hydroxyuridine inhibition with uridine.

It is evident from Figure 5 that protein synthesis is not *directly* involved in MF being separated from it in time. Furthermore, it appears that DNA synthesis is not involved in MF; little DNA synthesis is observed here until the MF process is complete. Since DNA synthesis commences at about the same time that the maximum amount of MF is attained in these experiments, the possibility exists that the initiation of DNA synthesis may limit further MF. We have no evidence to suggest that this is necessarily the case.

The Effect of Certain Analogues on Mutation Induction.—Utilizing the "EMB color" mutants of *E. coli* strain B, we previously have demonstrated[3] that the uridine analogue, 5-hydroxyuridine (5-HU), promotes a marked decline in mutation frequency with postirradiation incubation. However, this 5-HU promoted decline in mutation frequency does not start immediately but only after incubation of about 30 minutes and appears to be correlated in an inverse manner with the mutation fixation process. In view of the above results which correlate mutation fixation with RNA synthesis, it appeared possible that 5-HU may be exerting its effect through RNA synthesis. The plausibility of this hypothesis is demonstrated in Figure 6, which shows that the decline in mutation frequency (tryptophan reversion of *E. coli* strain WP2) involves a lag of some 20 minutes followed by decline in mutation frequency which is complete at 60 minutes. The initiation of the decline in mutation frequency in the presence of 5-HU is correlated with the initiation of RNA synthesis and is complete at about the time the relative amount of RNA has doubled. Protein synthesis begins only after a considerable degree of mutation frequency decline has occurred. Furthermore, DNA synthesis is initiated after the 5-HU promoted decline is complete. Thus the decline in mutation frequency in the presence of 5-HU can be correlated with RNA synthesis in the presence of 5-HU. It compares with the above described correlation of MF with RNA synthesis (in the absence of 5-HU mutation fixation begins with initiation of RNA synthesis and is complete at the time RNA synthesis has approximately doubled, Figure 5). A plausible hypothesis to account for this 5-HU effect is that 5-HU is incorporated into the RNA, thus producing "*nonfunctional*" RNA. Although such incorporation has not been demonstrated, this possibility must be entertained as the most plausible explanation of the decline in mutation frequency promoted by 5-HU and may be considered as support for the concept that synthesis of RNA involves incorporation of the "mutagenic substrate."

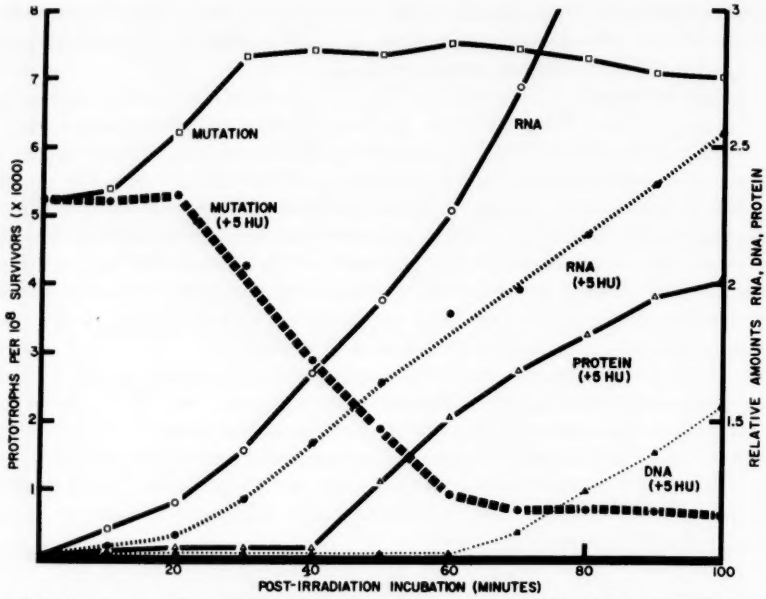

FIG. 6.—A comparison of UV induced mutation to prototrophy of the tryptophan requiring auxotroph, *E. coli* strain WP2, with and without postirradiation incubation in the presence of 5-hydroxy-uridine (5-HU) at 5 mg per 100 ml. The incubation medium was minimal medium plus casein hydrolysate, 2 mg per ml, and *dl*-tryptophan, 0.2 mg per ml. The relative increase of RNA in the absence of 5-HU and RNA, DNA and protein in the presence of 5-HU are also shown. No significant modifications of the level of survival were observed with incubation.

That protein synthesis may be involved in the mutation induction process must be considered. The demonstration by Schwartz and Strauss[8] of decline in the mutation frequency when *E. coli* strain WP2 is incubated in the presence of tryptosan (a tryptophan analogue which is incorporated into protein) lends support to this hypothesis. These workers did not present the kinetics of this decline in relation to protein synthesis, but it seems possible that the decline is mediated through formation of "nonfunctional" or "rejected" protein through incorporation of tryptosan (as they suggest) subsequent to mutation fixation. If protein thus formed were intimately involved with RNA in replication of genetic DNA (for example, in the formation of ribonucleoprotein) then "nonfunctional" protein might prevent utilization of the corresponding RNA in DNA replication. In this manner formation of "nonfunctional" protein might be expected to produce a decline in mutation frequency (in much the same way that 5-HU incorporation into RNA might be expected to cause a decline) through production of a nonfunctional ribonucleoprotein moiety thus using up the mutagenic substrate.

Discussion.—We have previously presented[3] an experimental hypothesis for UV-induced mutation in bacteria which suggests that mutation may be exerted through the macromolecular synthetic sequences involved in gene (DNA) replication. The experiments reported here lend further support for the plausibility of this hypothesis and also verify in a second mutational system (reversion from the tryptophan re-

quirement in *E. coli* WP2) some of the observations we have previously reported[3] for the "EMB color" mutations of *E. coli* strain B. The close correlation between RNA synthesis and "mutation fixation" in these UV exposed cultures is striking and suggests that mutations are "fixed" through RNA synthesis which may be intermediate in the replication of genetic DNA.

Stent[11] has recently presented a modification of the Watson-Crick scheme for DNA replication in which the double stranded helical DNA molecule serves as a template for synthesis of a nucleoprotein molecule consisting of one ribonucleic acid strand and a protein backbone. He suggests that the building blocks, or monomers, for the synthesis of the third chain could be an amino acid-ribotide complex, so that the growth of a polypeptide backbone chain could accompany the growth of the polyribotide chain. The genetic information of the parental DNA double helices would now reside in a single RNA chain. The mechanism for synthesis of replica DNA would be the inverse of that by which the single RNA strand has been formed by the parental DNA molecule. Thus a complementary pair of deoxyribonucleotides would be attracted by the single RNA base through the formation of specific hydrogen bonds. This would be followed by polymerization into DNA. The similarity of our experimentally formulated scheme for UV induced mutation and gene replication to Stent's theory is obvious.

The hypothesis for gene replication and UV-induced mutation previously presented[3] and a discussion of the relevance of the findings reported in this paper to the hypothesis follow:

(*1*) "*The principal mutagenic effect of ultraviolet light is to modify ribonucleic acid precursors present in the cell at the time of radiation exposure.*"

The evidence suggests that the mutagenic nucleic acid precursors are UV-modified RNA pyrimidines. We have very little concrete information concerning the nature of the proposed mutagenic nucleic acid precursors. Sinsheimer[12] has shown that exposure of uridylic acid or cytidylic acid to UV results in the formation of stable and unstable products. None of the other ribonucleotides or deoxyribonucleotides demonstrate this property. We have shown that cytidine and uridine are the effective compounds in the production of the mutation frequency increase with preirradiation incubation[1] and that these compounds are the only nucleic acid precursors decreasing mutation when added after irradiation, presumably through competition with the radiation produced mutagenic nucleic acid precursors.[3] Thus the irradiation products described by Sinsheimer could be the mutagenic agents involved.

(*2*) "*These modified precursors are temporarily stabilized in the cell by an enzymatic process dependent on a source of amino acids (mutation stabilization). If the precursors are not stabilized, they are rapidly removed (or lose their capacity to effect mutation) by a second enzymatic process (mutation frequency decline).*"

The process of "mutation stabilization" involves a complex supply of amino acids which must be supplied to the cells immediately after UV irradiation at a relatively high level. Involvement of the amino acids and the apparent involvement of nucleic acid precursors in the "mutation stabilization" process suggested to us that "mutation stabilization" may involve the formation of amino-acid-nucleotide complexes.[3] The possibility exists that the process of "mutation stabilization" actually involves the specific association of amino acid-nucleotide monomers with

the DNA double helix as proposed by Stent.[11] If "mutation stabilization" involves "templating" of radiation modified RNA precursors in interaction with amino acids, then the "mutation frequency decline" process might involve enzymatic conversion of these precursors to metabolites not utilizable in the templating process, at least in the direct line of gene replication.

(3) *"Mutation is exerted through the process of incorporation of modified nucleic acid precursors with RNA synthesis resulting in modification of the RNA."*

The striking correlation between RNA synthesis and "mutation fixation" in UV-exposed cultures lends support to the view that mutation is "fixed" through RNA synthesis. That "mutation fixation" as measured by photoreversal is closely correlated here lends credence to the idea that "mutation fixation" is a manifestation of the macromolecular synthetic event directly involved in establishment of that change which constitutes mutation. The correlation of mutation frequency decline produced by 5-hydroxyuridine with RNA synthesis is further support for this view, since this analogue could become incorporated to produce "nonfunctional" RNA. This would use up the mutagenic substrate in formation of a product which would then be useless for gene replication.

We cannot determine at present the significance of the fact that the maximum level of "mutation fixation" is attained at the time that the cellular RNA has doubled. As one possible explanation, the level of mutation induction may be limited by the amount of "modified nucleic acid precursor" which can be accommodated on templates preliminary to synthesis of that portion of RNA involved in gene replication. Presumably all UV-modified precursors not so accommodated on templates are enzymatically attacked by the same metabolic process which is responsible for "mutation frequency decline." This does not mean that all the cellular RNA synthesized during the mutation fixation period is involved in mutation induction (or gene replication). Rather the RNA synthesis involved may be minor but correlated with gross RNA synthesis in the cell under our experimental conditions.

The amino acid analogue data of Schwartz and Strauss[8] lend support to the view that protein synthesis is involved in the mutation induction process. The formation of a ribonucleoprotein chain through the initial polymerization of the nucleotide monomers into RNA and subsequent synthesis of the protein backbone (as proposed by Stent) may be the intermediate step in mutation induction (and gene replication).

(4) *"It may be that RNA and protein synthesis are involved in replication of DNA, and changes in RNA specificity result in corresponding changes in genic DNA."*

The evidence presented above suggests that the initial mutagenic event following alteration of the nucleic acid precursors by UV involves RNA synthesized from these precursors. The possibility exists that RNA synthesis is intermediate to synthesis of DNA. Thus RNA (and possibly protein) may actually mediate transfer of "information" from parent to daughter genetic DNA.

The weakest link in our chain of evidence is whether or not the genetic character studied and the mutations induced reside in the genetic DNA. We hope through bacterial mating techniques to determine more satisfactorily whether the mutation induced ultimately resides in the daughter genetic DNA as would be predicted from the above hypothesis and *not* in the parental DNA.[13]

If we accept the view that the paired nucleotide sequence in the double helix of DNA constitutes the basis for "coding" in genetic specificity, that replication of DNA involves the conservation of this specific sequential relation and if we further adopt the view that the ribonucleoprotein chain is intermediate in genetic DNA replication, then a plausible hypothesis for mutation based on the change in the composition of a single nucleotide pair unit in the daughter DNA may be advanced. It would be necessary to assume that the UV-modified RNA nucleotide has either lost its ability to direct the insertion of a specific nucleotide pair into the daughter DNA or that this ability has been modified so that the "wrong" nucleotide pair will be inserted in the daughter DNA in relation to the parental DNA. Thus the insertion of a modified nucleotide in the intermediate RNA chain could lead to synthesis of daughter DNA modified by one nucleotide pair unit.

It is thus plausible that mutation may actually consist of a change in one nucleotide pair unit of DNA. The findings of Ingram[14] with sickle cell and "C" hemoglobin (abnormal proteins genetically determined by a change in one hereditary unit in man) suggest that the mutations involved may influence only the substitution of one amino acid for another in the hemoglobin peptide chains. It is therefore conceivable, if this proves general in the genetic modification of protein, that the "one gene-one enzyme hypothesis" may have a "one nucleotide pair unit-one amino acid" corollary, in so far as the mutational modification of genetic and enzymatic specificities is concerned.[15]

It is evident that involvement of an intermediate ribonucleoprotein in DNA replication not only would provide a fortuitous mechanism for genetic change but could provide for the immediate heterogenetic function of the gene, in that the ribonucleoprotein might also serve as the basis for gene action in formation of specific enzymes. This hypothesis is attractive both from the functional viewpoint and from the viewpoint of evolution of heterogenetic activity from the replicative process, such as must have taken place in the early stage of evolution of living systems.

The authors wish to acknowledge the capable technical assistance of Miss Ethelyn Lively, Mr. James Gowan and Miss Fay Doyle. Valuable discussions with Dr. David Ezekiel are acknowledged gratefully.

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[11] Stent, G. S., *Advances in Virus Research*, **V,** 95 (1958).

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[13] We wish to thank Dr. R. L. Sinsheimer of the California Institute of Technology for his interest and suggestions along this line.

[14] Ingram, V. M., *Nature*, **180**, 326 (1957); Hunt, J. A., and V. M. Ingram, *Nature*, **181**, 1062 (1958).

[15] It should be made clear that here "mutation" does not include gross or direct effects of mutagenic agents on DNA or chromosomes to produce deletions, inversions or other abnormalities which render a particular gene inactive or modify its phenotype effect. It seems reasonable for purposes of *study of the induction process* to divide "mutations" into at least two classes: (1) those mutations probably involving one DNA nucleotide pair and exerted through the process of gene replication and (2) those mutations involving one or *more* nucleotide pairs exerted finally through some physical or chemical action on DNA (or RNA) and *not* through gene replication. In the case of UV-induced mutation it appears that the former by far exceed in number the latter (if they occur at all). Further this is not to suggest that one nucleotide pair may somehow "code" a particular amino acid but rather that a change in the nucleotide composition of the nucleotide pair unit in a particular *sequence* may lead to a change of one amino acid originally specified by the particular sequence involved to another amino acid.

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*TRANSDUCTION BY STAPHYLOCOCCAL BACTERIOPHAGE**

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Transduction of genetic material has been reported previously for *Salmonella* and *Escherichia coli* bacteriophages.[1] It is the purpose of this communication to call attention to gene transfers accomplished by staphylococcal bacteriophage.

Materials and Methods.—Preliminary studies suggested that among the staphylococcal typing phages examined,[2] NCTC 8406 (phage 53) and its propagating bacterial strain NCTC 8511 would be suitable for study of gene transfer between staphylococcal cells. Phage 8406 forms plaques well on 8511 and an appreciable number of the 8406–8511 complexes result in lysogeny of the 8511 cells. Such lysogenic cells formed are subsequently induced to produce phage by ultraviolet radiation.[3] Because of these advantageous features a variety of bacterial mutants was obtained in the 8511 line, and selected for further study.

Cultures were grown in 10 ml volumes of Difco nutrient broth on a rotating (28 rpm) circular test tube rack at 37C. Cell clumping which can be a problem with staphylococci, was controlled by allowing the cultures to grow in a stationary position for 10–12 hours, followed by 4–5 hours of growth in the rotating rack. This procedure usually produces cultures with about 2×10^9 cells per ml in which 50 per cent of the cells are single, 46 per cent are either in pairs or are single cells about to divide, and the remaining 4 per cent occur chiefly as clamps of 3–4 cells. Ultraviolet inactivation of these cultures is not appreciably different from a one-hit process to a 1 per cent survival level.

Phage lysates were prepared by UV induction of cultures lysogenic for 8406 phage. Cultures were removed from the rotator and suspended in saline or phosphate buffer solutions at a density of about 5×10^8 cells per ml and irradiated with ultraviolet to give 1.0–0.1 per cent survival. Concentrated nutrient broth (10×) was

added (1 volume/10 volumes) to the irradiated suspension and the mixture rotated for 4–6 hours at 37C, after which it was left on the bench at about 20 C for 16 hours. During this latter period the cultures usually cleared. Cleared cultures had from 2×10^9 to 10^{10} plaque particles per ml on 8511. The lysates were sterilized by filtration through Mandler filters.

Transduction experiments were performed by mixing phage with phage-sensitive cells at multiplicities of one phage or less per cell and allowing the mixture to incubate at 37C for 5–25 minutes, during which time 95 per cent of the added phage adsorbed to the cells. Samples of 0.1–0.8 ml were added to 2 ml volumes of melted 0.6 per cent agar (50C) and poured on either nutrient agar or on agar indicating acid production from carbohydrates.[4] Selection was made for transduction of genes for drug resistance characters,[6] and to accomplish this it was necessary to overcome 4–6 hours of phenotypic delay.[5] The agar pour platings were overlayed routinely with melted 0.6 per cent agar containing drug within one hour of plating to serve as a control, and after four hours, to enumerate transductions.

Results.—Transduction of the genes for resistance to streptomycin and novobiocin was observed. The data given in Table 1 show that streptomycin-resistant clones

TABLE 1

TRANSDUCTION OF THE GENES FOR RESISTANCE TO STREPTOMYCIN AND NOVOBIOCIN[6]

Expt.	Phage Source	Amount of Phage $\times 10^9$	Drug-Resistant Clones Produced S^r	N^r
50	N^s	7.7	...	0
	N^r	8.8	...	680
51	N^sS^s	1.9	0	20
	N^rS^r	4.0	1,210	1,100
78	S^s	3.9	0	
	S^r (1)	4.6	290	
	S^r (2)	3.1	330	
TG-61	N^s	2.6	...	10
	N^r	8.5	...	300

are formed only when streptomycin-resistant cultures (S^r) are the source of phage. Larger numbers of novobiocin-resistant clones (N^r) were found on plates with phage from N^r sources than from novobiocin sensitive (N^s) sources. Since platings containing no phage have small numbers of N^r, we believe the source of the novobiocin-resistant clones on the platings from N^s sources to be spontaneous reversions.

The number of drug-resistant clones produced by transduction is proportional to the amount of phage employed (Fig. 1).

The phenotypic expression of the tranduced S^r genes is accomplished in 4–6 hours, while approximately 50 per cent of the transduced N^r genes appear to achieve phenotypic expression immediately upon entering the cell (Fig. 2). The immediate expression of N^r would suggest that N^r is dominant to N^s, however the delayed N^r require some other explanation, such as delay in penetration of the cell. It is doubtful that this delay would be of 4–5 hours' length. Alternately, the behavior of the transduced N^r genes may indicate that novobiocin is a slowly acting drug, requiring 3 hours for its killing effect. During such a time the phenotypic expression of transduced resistance genes might be achieved.

The data indicate that approximately one phage particle in 10^7 to 10^8 carries a gene for streptomycin or novobiocin resistance.

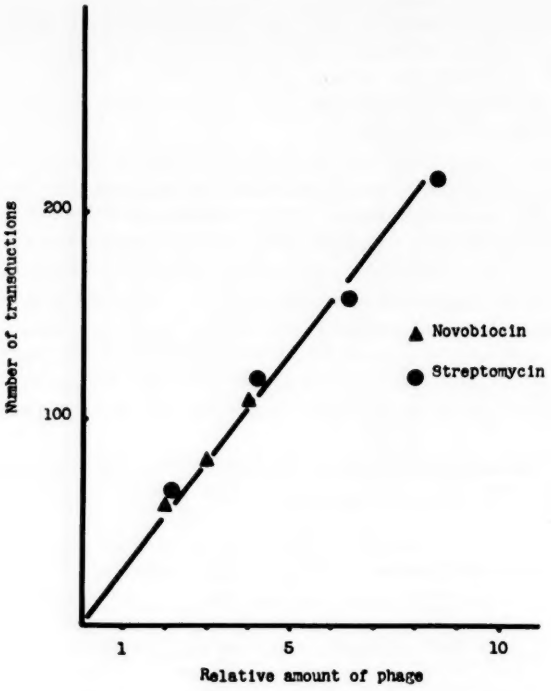

FIG. 1.—Proportionality between number of transductions and amount of phage. Different lysates: relative amount 1 for streptomycin equals 4.5×10^8 phage particles; relative amount 1 for novobiocin equals 1.7×10^9 phage particles. The frequencies of transduction are: S^r, one per 2×10^7; N^r, one per 6.8×10^7.

The data given also indicate that about one cell in 10^7–10^8 exposed to a phage preparation yields a cell of changed genotype. Such a low frequency virtually eliminates the detection of coincidental transductions and any clones altered for more than a single genetic character can be considered to indicate the transduction of closely linked genes. Genetic linkage has been looked for by using multiply marked stocks, for example: $S^rN^rMan^-Lac^+$ (donor phage source) $-X$ (applied to:) $S^sN^sMan^+Lac^-$ (phage-sensitive recipient cells).[6] The S^r or N^r produced by transduction were examined for changes of the other distinguishing genetic markers. No evidence for linkage has been found between any of the following markers: *S*, *N*, *E*, *Lac*, *Man*, with at least 300 transductions per test.

Of 462 transductions, 461 were found lysogenic with respect to the parent NCTC 8511 strain, evidence that phage is the vector or co-vector of genetic transfer. The remaining culture does not produce a plaque-forming phage and is non-sensitive to exogenous phage and may therefore carry a defective prophage. The complexity of the situation with regard to phage deserves additional comment. The host culture NCTC 8511 and its derivatives are lysogenic for a phage which

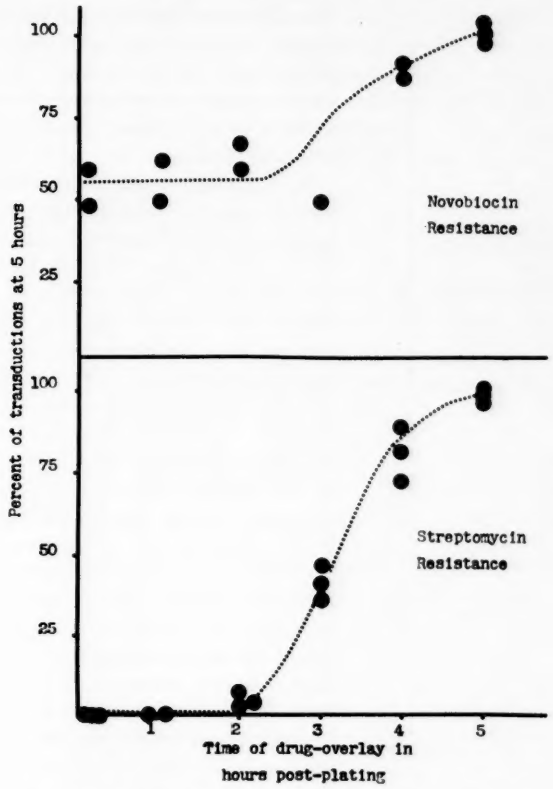

FIG. 2.—Phenotypic expression of transduced genes. The maximum overlay time was 5 hours post plating. At this time approximately 90 per cent of transductions have been phenotypically expressed.

attacks NCTC 8325, the propagating strain of typing phage 47.[7] There is good evidence also for a third phage in our lysates.[8] These phages have been found at low titers in our transducing lysates (10^{-4}–10^{-6} of the phage-forming plaques on 8511). Direct test of the phage attacking NCTC 8325 failed to show transduction of streptomycin resistance between cells of the 8511 line by this phage. Because of the low incidence of these phages in our lysates, and the negative results with one of them in transduction experiments, we ascribe our transduction to the major phage constituent of our lysates, phage 8406 (53).

In a transduction system in *E. coli* the formation of partially diploid clones (heterogenotes), as a result of transduction, was noted.[9,10] UV-induction of these heterogenotic cultures yielded lysates giving high frequency of transduction, because most phage particles carried bacterial genetic material. Examination of the transductional clones formed by staphylococcal bacteriophage failed to show a heterogenotic condition for either Man or Lac. Lysates of 4 N^r clones and 4 S^r

TABLE 2

DOUBLY DRUG-RESISTANT CLONES PRODUCED IN MIXED GROWTH OF SINGLY RESISTANT MUTANTS OF STAPHYLOCOCCI[6]

Expt.	Genotypes in Mixed Growth Strain 1	Strain 2	Resistance Markers Selected	Non-selected Markers	Non-selected Markers Observed	Suspected Reversion of Strain*
1	$N^sS^rLac^-Ly^+$	$N^rS^sLac^+Ly^-$	N^rS^r	*Lac, Ly*	37 Lac^-Ly^+	1
2	$N^sS^rLac^-Man^+Ly^+$	$N^rS^sLac^+Man^-Ly^-$	N^rS^r	*Lac, Man, Ly*	58 $Lac^-Man^+Ly^+$	1†
3	$S^rE^rLac^-Man^+Ly^+$	$S^sE^rLac^+Man^-Ly^-$	S^rE^r	*Lac, Man, Ly*	4 $Lac^-Man^+Ly^+$	1
					6 $Lac^+Man^-Ly^-$	2
4	$S^rE^rLac^-Man^+Ly^+N^s$	$S^sE^rLac^+Man^-Ly^-N^r$	S^rE^r	*Lac, Man, Ly, N*	18 $Lac^-Man^+Ly^-N^s$	1
					2 $Lac^+Man^-Ly^+N^r$	2
5	$N^rE^rLac^+Man^-S^s$	$N^sE^rLac^-Man^+S^r$	E^rN^r	*Lac, Man, S*	35 $Lac^-Man^+S^r$	2
					4 $Lac^+Man^-S^s$	1

* The non-selected markers are derived from one *or* the other strain in the growth mixture and therefore suggest that spontaneous reversion was the mechanism by which the doubly drug-resistant strains were formed. For example, in experiment 1, $N^sS^rLac^-Ly^-$ yields $N^rS^rLac^-Ly^+$ by spontaneous reversion of N^r.

† Three experiments, in one of which inclusion of *P. coli* failed to influence the number or kind of doubly resistant staphylococci produced in the mixture.

clones produced by transduction gave a low frequency of transduction of N^r and S^r (one transduction per 10^7–10^8 phage). Staphylococcal bacteriophage transductions therefore appear to resemble those found in Salmonella by Zinder and Lederberg.

It is possible with the genetically labeled strains used for transduction studies to test for sexual recombination between cells of the NCTC 8511 strain. This can be done by selection for double drug resistance, each of the parent cells contributing one resistance gene, and by the use of two or more unselected genetic markers:[11] $N^rS^sMan^+Lac^-$ crossed with $N^sS^rMan^-Lac^+$ yields $N^rS^rMan^-Lac^-$ and $N^rS^rMan^+$ Lac^+ combinants for double drug resistance (selected) in which recombination for the fermentation markers (unselected) has occurred. Using various combinations of *N*, *S*, *E*, *Lac*, *Man* and *Ly*, we have made limited tests for sexual recombination in our strains. Although strains doubly resistant to drugs were obtained, these were always non-recombinant for unselected markers (Table 2). The most plausible origin of these doubly resistant strains is spontaneous mutation. In support of this explanation was the association between the occurrence of doubly resistant strains and the mutabilities of the loci for drug resistance. Qualitatively the mutability order is $S < E < N$. Doubly drug resistant clones were obtained most frequently in combinations of the more mutable loci (*N*, *E*) and the clones most often resembled the more mutable "parent."

Discussion.—The evidence that has been presented indicates that staphylococcal bacteriophage preparations (of typing phage 53) can transfer staphylococcal genetic material. The limitations on the number of genetic markers found transferred is owing to the lack of genetic factors for which selection can be exercised. Since the nutrition of our strains is nearly defined,[8] it is expected that requirements for amino acids and vitamins, and the utilization of carbohydrates will become available shortly for further study of this transduction system. It is hoped that the medically

important staphylococcal characteristics such as hemolysin and coagulase production will become available for analysis, either directly through selection or by means of linkage to other selectable markers. More details of the above and the results of further experiments will be reported elsewhere.

Summary.—Genetic transduction by staphylococcal bacteriophage has been demonstrated. About one phage particle in 10^7–10^8 transfers the genes for resistance to streptomycin and novobiocin.

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[6] Symbols are *N*, Novobiocin; *S*, Streptomycin; *E*, erythromycin; *Lac*, lactose fermentation; *Man*, mannitol fermentation; *Ly*, lysogenicity. Superscripts: *r*, *s*, resistant and sensitive respectively; $^+$, $^-$, ability to ferment and inability to ferment carbohydrates, except in the case of *Ly*, where $^+$ signifies lysogenic, $^-$ signifies nonlysogenic and sensitive to exogenous phage. The parent staphylococcal strain is inhibited by 1 mcg/ml streptomycin and 0.05 mcg/ml novobiocin. The resistant strains are not inhibited by 25 mcg/ml and 2 mcg/ml, respectively. The concentration of the drugs in the overlay agar were: *S*, 100 mcg/ml; *N*, 20 mcg/ml.

[7] Unpublished observations and a personal communication from Dr. P. M. Rountree.

[8] Unpublished observations, John Mann, this laboratory.

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*ABERRANT TETRADS IN SORDARIA FIMICOLA**

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In an earlier paper[1] describing three ascospore color mutants in the homothallic pyrenomycete *Sordaria fimicola*, the rare occurrence of unusual tetrads with unexpected ratios of wild-type and mutant ascospores was noted for all three loci. In the course of further studies of two of these mutant loci, a number of asci showing aberrant tetrads were isolated and analyzed in an effort to obtain a clearer idea of the mechanism responsible for their occurrence. Such tetrads in yeast had previously been explained by Lindegren[2] as resulting from "gene conversion" in a heterozygote, usually involving a change of the dominant gene to its recessive allele. Later, Mitchell[3] reported similar abnormal segregations for the pyridoxine locus in *Neurospora*, which she explained as probably resulting from double replication.

A similar explanation is offered by Case and Giles[4] in an interpretation of irregular segregation at the *pan-2* locus in *N. crassa*. These authors use the term "copy choice" suggested earlier by Lederberg.[5] Glass[6] refers to the phenomenon as "transreplication," a term that seems adequate for purposes of this discussion.

Procedure.—All asci described here were derived from crosses between cultures with the wild-type allele for the production of dark ascospores and either of two different ascospore color mutants, hyaline-spored (*h*) or gray-spored (*g*). All crosses were also heterozygous for one other mutant factor. Perithecia from the line of contact between paired cultures were pressed under a cover slip, after which the hybrid clusters of asci were removed to an adjacent drop of water on the slide and flattened out under a cover slip. These asci were then examined for aberrant tetrads showing disproportionate numbers of wild-type and mutant spores. A cluster containing such an ascus was removed to a dish of agar and the desired ascus isolated and dissected.

Results.—An analysis of 23 abnormal asci was made (Table 1). The first 12 asci were derived from crosses heterozygous for the *h* locus, which is situated at about 32.5 crossover units from the centromere, and for one other locus—d_1, d_2, or *a*-2. Unpublished data on the last three loci show that they are unlinked, but that d_1 is linked to *h* with a distance of about 28 crossover units between them. Both d_1 and d_2 cause dwarfness of cultures, while *a*-2 is recessive for ascus abortion. In asci heterozygous for the *a*-2 locus, the *a*-2 ascospores develop normally. The d_1 locus is only a few crossover units from the centromere, while d_2 is about 14.5 units out on its chromosome arm, and *a*-2 is far out on its chromosome arm. The *h* factor, in addition to causing hyaline spore color, is lethal for ascospore germination.

Asci 1–7, 10, and 11 have a ratio of $6h+:2h$ ascospores. With respect to the

TABLE 1

ASCI OF *S. fimicola* SHOWING ABERRANT SEGREGATION FOR SPORE COLOR*

Cross	Ascus No.	Spore Color Ratio	Spore No. and Genotype 1	2	3	4	5	6	7	8
$h+d_1+Xhd_1$	1	6+:2-	+−	+−	+−	+−	++	++	(−+)	(−+)
"	2	"	(−−)	(−−)	+−	+−	++	++	++	++
"	3	"	(−−)	(−−)	+−	+−	++	++	++	++
$h+d_1Xhd_1+$	4	"	(−+)	(−+)	++	++	+−	+−	+−	+−
"	5	"	+−	+−	+−	+−	++	++	(−+)	(−+)
"	6	"	+−	+−	+−	+−	++	++	(−+)	(−+)
"	7	"	+−	+−	+−	+−	++	++	(−+)	(−+)
"	8	5+:3-	(−+)	(−+)	++	++	+−	+−	(−−)	**+**−
"	9	"	(−+)	(−+)	(−+)	**+**+	+−	+−	+−	+−
$h+d_2Xhd_2+$	10	6+:2-	(−−)	(−−)	+−	+−	++	++	++	++
$h+a\text{-}2Xha\text{-}2+$	11	"	++	++	(−+)	(−+)	+−	+−	+−	+−
"	12	5+:3-	(−−)	(−−)	++	++	+−	+−	(−+)	**+**+
$gstm+Xg+stm$	13	"	+−	+−	−−	**+**−	−+	−+	++	++
"	14	"	**+**+	−+	+−	+−	−+	−+	+−	+−
"	15	"	−+	−+	**+**+	−+	+−	+−	+−	+−
"	16	6+:2-	+−	+−	+−	+−	−+	−+	++	++
"	17	"	+−	+−	+−	+−	−+	−+	++	++
$g+a\text{-}1+Xga\text{-}1$	18	"	+−	+−	+−	+−	−+	−+	++	++
"	19	"	++	++	+−	+−	+−	+−	−+	−+
"	20	"	(−+)	−+	++	++	+−	+−	+−	+−
"	21	5+:3-	−−	**+**−	+−	+−	−+	−+	++	++
"	22	2+:6-	−−	−−	−−	−−	++	++	−+	−+
"	23	3+:5-	+−	+−	**−**−	+−	−+	−+	−+	−+

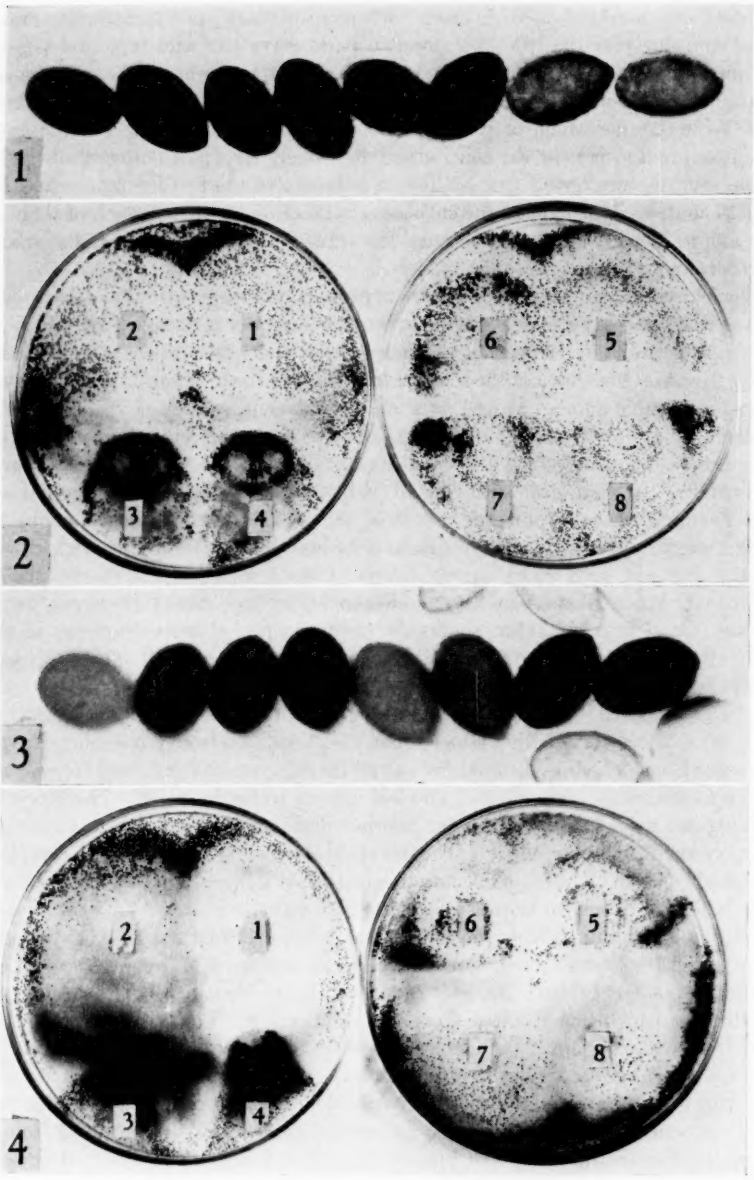
* Unrecovered progeny in parentheses. The *h* allele is lethal for ascospore germination. The aberrant alleles, where identifiable (in 5:3 asci), are indicated in bold type. The symbols + and − signify wild type and mutant alleles, respectively; the first symbol under each spore genotype refers to a spore color locus.

second locus involved in each cross, two pairs of dark spores carry the mutant allele and one pair the wild-type, or two pairs carry the wild-type and one pair the mutant allele. In other words, one pair of spores which would be expected to contain the *h* allele, has instead the wild-type allele at that locus. It is not possible to determine which of the three pairs is the aberrant one. Since *h* and d_1 are linked and d_1 is near the centromere, it is likely that in a majority of the first seven asci, the converted pair is the one adjacent to the hyaline pair. Asci 8, 9, and 12 contain ascospores in the surprising ratio of $5h+:3h$. In each of them it is possible to identify the spore carrying the transreplicated locus, since its adjacent partner carries the *h* allele and is hyaline.

Asci containing an excess of *h* spores in proportion to *h*+ were also observed, but it was not feasible to dissect them for further analysis in view of the failure of *h* spores to germinate. However, an examination of 2,700 hybrid asci was made in order to obtain some idea of the relative frequency of the different types of abnormal tetrads. Of this number 11 asci, or about 0.4 per cent, contained visibly abnormal tetrads. Six showed a $6h+:2h$ segregation, five of these having the six dark spores in a series and one having the arrangement $2h+:2h:4h+$; five asci showed a ratio of $2h+:6h$, four of these having all six hyaline spores in an uninterrupted series and one having them in the order $2h:2h+:4h$. No 5:3 segregations were observed in this group, and in general they appear to be less common than 6:2 asci. It would appear from the data that transreplication of the *h* locus, if that is the phenomenon involved, is about equally common in either direction. However, in view of the fact that rare hyaline spores are produced probably as the result of other aberrations in nuclear division, it is not possible to be sure that all extra hyaline spores have developed in like manner.

The gray-spored locus (*g*) offers a better approach to this problem, since gray and wild-type spores are equally viable. Asci 13–23 resulted from crosses heterozygous for the *g* locus, which is situated far out on its chromosome arm, and for one additional factor either *stm* or *a*-1. The loci appear to be unlinked. The crosses involving *stm* are all homozygous for another mutant locus *st*-1, which causes near sterility in cultures carrying it. The *stm* allele converts *st*-1 cultures into completely self-sterile ones.[7] The *a*-1 allele has an effect exactly like *a*-2 in causing ascus abortion. There appears to be no linkage of *g* with either *stm* or *a*-1. The *stm* locus is about 6 crossover units from the centromere, while *a*-1 is far out on its chromosome arm. Asci 16–20 show a segregation of $6g+:2g$, and in all of them the second locus segregated in a 4:4 ratio. Again it is not possible to identify any of the three pairs as the one which has resulted from transreplication. Figure 1 shows an ascus of this type and Figure 2, the eight cultures derived from spores of the same ascus (Ascus 19, Table 1). All of these crosses are heterozygous for unselected factors affecting growth rate and perithecial distribution. This is of some advantage to the analysis, since it offers another way of recognizing spore pairs resulting from the third division in the ascus and insures against any misinterpretation due to disorderly spore arrangement.

Asci 13–15 and 21, which show a ratio of $5g+:3g$, are among the most revealing ones obtained. In all of them it is possible to identify the spore carrying the transreplicated locus, since it is a dark spore and its sister spore is gray. Figure 3 shows such an ascus and Figure 4, the eight cultures obtained from it (Ascus 21, Table 1).



FIGS. 1–4.—Aberrant tetrads in *Sordaria fimicola*. Fig. 1, $6g+:2g$ ascus (Ascus 19, Table 1); Fig. 2, progeny on corn meal agar plates; Fig. 3, $5g+:3g$ ascus (Ascus 21, Table 1); Fig. 4, progeny on corn meal agar plates. (Spores in asci are numbered from left to right.)

It is obvious that the second spore in this ascus is the odd one. The cultures demonstrate another interesting feature, i.e., the culture derived from the odd dark spore is conspicuously darker than that derived from the gray sister spore. Our original gray-spored mutant produces a light, opaque mycelium, whereas, the wild-type produces a dark pigment, probably melanin. The difference is usually more pronounced in test tube cultures than on agar plates. In all of the $5g+:3g$ asci the unexpected dark spore gives rise to a pigmented mycelium. Also the dark spores in asci with a ratio of $6g+:2g$ produce pigmented colonies. Mycelial pigmentation is in part a pleiotropic effect of the *g* locus. Some of our unpublished data indicate that one or more closely linked loci may also be involved in mycelial pigmentation, but since this character is not a sharply defined one and since other unselected loci also modify the degree of pigmentation, we have not been able definitely to establish this. If such loci do exist, the data indicate that they may also undergo transreplication simultaneously with the *g* locus. However, it is not likely that this could be confirmed without the aid of closely linked markers with more clear-cut phenotypic effects.

Only two asci with a greater than normal number of gray spores were isolated from these crosses. They appear to be less common than the foregoing types. Ascus 22 shows a $2g+:6g$ segregation and ascus 23, a $3g+:5g$ ratio. The third spore of the latter ascus, which is obviously the odd one in the series and has apparently resulted from transreplication of the *g* allele instead of the normal duplication of the $g+$ allele, produced a mycelium that was lighter than that derived from the fourth, or sister, spore carrying the $g+$ allele. Otherwise the two cultures were alike in growth rate and appearance, being slower than any of the others. Abnormal tetrads resulting from crosses heterozygous for the *g* locus are distinctly less common than those from crosses heterozygous for the *h* locus. Although our observations do not at this time permit an accurate statement regarding their frequency, it is estimated that not more than one ascus in 800–1,000 show such aberrant tetrads.

The genotypes of all spore progeny from abnormal tetrads, when examined at maturity or in crossing tests, proved to carry the color factor assigned to them in direct examination of the asci. A cross between the culture derived from the odd dark spore (the fourth) in ascus 13 and a *g* culture resulted in further transreplication among the hybrid asci. Therefore, the transreplicated locus behaves in every way like the typical wild-type locus.

Discussion.—In an earlier paper[1] it was reported that an examination of many thousands of asci of both ultraviolet irradiated as well as non-irradiated cultures of each of the spore color mutants failed to produce any evidence of back mutation at the color loci. Although a much larger number of asci have now been observed over an additional 4-year period, there is still no evidence of back mutation at these loci in pure mutant cultures. It is also clear from the evidence that the aberrant asci described here cannot be the result of typical crossing over, since there are no reciprocal products of crossing over among the progeny. It is equally obvious that the odd ratios have not resulted from nuclear degeneration in the ascus followed by compensating nuclear divisions among the surviving genotypes, since the spores may be readily paired off by means of a second genetic marker in each cross and by unselected factors affecting growth rate. The most logical explanation would

appear to be one based on a transreplication or copy choice mechanism occurring at meiotic prophase while the chromatin strands are duplicating.

The most significant asci found are those with a 5:3 ratio for spore color. While it is possible to explain 6:2 asci on the basis of transreplication within the conventional 4-strand model at meiotic prophase, this is not feasible with the 5:3 asci. Ris[8] has obtained evidence from electron microscopy that chromatids are further subdivided into half-chromatids or quarter-chromatids, depending upon the organism and varying states of the chromosomes in the same organism. Taylor[9] proposes that each chromatid is composed of two functional subunits. He has anticipated the transreplication mechanism that would lead to the production of a 5:3 ratio (see his Fig. 7, *B*). The 6:2 ratio is explained on the basis of at least one breakage among the substrands in conjunction with transreplication off both substrands of the chromatid being copied at a particular locus (see his Fig. 7, *C–E*). This interpretation would readily account for the results which we have obtained. Our 5:3 asci support the concept of the chromatid as being composed of two functional subunits.

The aberrant asci reported here could actually be more accurately referred to as octads. Certainly, the 5:3 pattern would not be readily detected in an ordinary tetrad of four meiospores, nor in an 8-spored ascus if the ascospores were isolated in pairs. The third division in the ascus is required for the separation of the transreplicated substrand from its normal partner. In a 4-spored yeast, for example, a spore carrying a heterogeneous chromatid would most likely give rise to mixed progeny and the data interpreted as resulting from a binucleate, heterokaryotic spore, back mutation in the developing colony, or contamination. The phenomenon might also contribute interesting effects elsewhere. For example, some of the reports of self-fertility among single-spore isolates of heterothallic ascomycetes and of spontaneous dikaryotization in single-spore cultures of basidiomycetes might have resulted from this mechanism.

Eight different ascospore color mutants of *S. fimicola* have now been produced in our laboratory. It would be ideal to obtain other mutant loci closely linked on either side of a color locus so that more detailed information could be obtained on the area transreplicated.

Summary.—A study of 23 asci showing aberrant segregation for ascospore color revealed that some mechanism other than crossing over, back mutation, or irregularities in nuclear survival is responsible for their occurrence. It is believed they have resulted from transreplication, by which a locus is copied more than the normal number of times during replication at meiotic prophase. The occurrence of 5:3 ratios in some asci lends confirmation to the hypothesis that each chromatid is composed of two functional subunits.

* Supported by NSF Grant G-2808 and NIH Grant E-2326.

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UNIQUE FACTORIZATION IN REGULAR LOCAL RINGS

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In this note we prove that every regular local ring of dimension 3 is a unique factorization domain. Nagata[4] showed (Proposition 11) that if every regular local ring of dimension 3 is a unique factorization domain, then every regular local ring has unique factorization.* Thus, combining these results we have that every regular local ring is a unique factorization domain.

Throughout this note R is a local ring with maximal ideal $\mathfrak{M}$. The definitions and notation follow those of Auslander and Buchsbaum.[1]

Proposition 1. *Let x be in $\mathfrak{M}$ and y in R such that $\mathfrak{a} = (x) : y$ satisfies the following conditions: (a) $hd\ \mathfrak{a} \leq 1$ and (b) x is not in $\mathfrak{M}\mathfrak{a}$. Then $\mathfrak{a} = (x)$ and x is not a zero divisor.*

Proof: Suppose x does not generate $\mathfrak{a}$. Since x is not in $\mathfrak{M}\mathfrak{a}$, there exist $a_1, \ldots, a_n$ in $\mathfrak{a}$ $(n > 0)$ such that $x, a_1, \ldots, a_n$ form a minimal generating set for $\mathfrak{a}$. Let R^{n+1} denote the direct sum of $n + 1$ copies of R, define $f: R^{n+1} \to \mathfrak{a}$ by $f(r_0, \ldots, r_n) = r_0 x + \sum_{i=1}^{n} r_i a_i$, and let $K = \operatorname{Ker} f$. Since $x, a_1, \ldots, a_n$ is a minimal generating system for $\mathfrak{a}$, we have that f is an epimorphism and K is contained in $\mathfrak{M}R^{n+1}$. From the exact sequence $0 \to K \to R^{n+1} \to \mathfrak{a} \to 0$ and the fact that $hd\ \mathfrak{a} \leq 1$, we have that K is R-free. Since $\mathfrak{a}$ is not principal, we have that $hd\ \mathfrak{a} = 1$ and thus $K \neq 0$.

Let $N_i = (t_{i0}, \ldots, t_{in})$ be a free basis for K over R $(i = 1, \ldots, m)$. Since a_1 is in $\mathfrak{a}$, we have that $ya_1 = -vx$ for some v in R. Let $V = (v, y, 0, \ldots, 0)$ and $T = (a_1, -x, 0, \ldots, 0)$. Then V and T are in K. Let $V = \sum_{j=1}^{m} r_j N_j$ and $T = \sum_{j=1}^{m} s_j N_j$. Now $xV = -yT$. Therefore we have that $\sum_{j=1}^{m} x r_j N_j = \sum_{j=1}^{m} -y s_j N_j$. Since the $\{N_j\}$ are a free basis for K over R, we have that $xr_j = -ys_j$ for all $j = 1, \ldots, m$. But $(x) : y = \mathfrak{a}$. Therefore we have that each s_j is in $\mathfrak{a}$. Since $T = (a_1, -x, 0, \ldots, 0) = \sum_{j=1}^{m} s_j N_j$, it follows that $-x = \sum_{j=1}^{m} s_j t_{j1}$. Therefore x is in $\mathfrak{M}\mathfrak{a}$ (since K is contained in $\mathfrak{M}R^{n+1}$) which contradicts the fact that x is not in $\mathfrak{M}\mathfrak{a}$. Thus $\mathfrak{a} = (x)$. The fact that x is not a zero divisor follows from the assumption $hd\ \mathfrak{a} \leq 1 < \infty$ and [2, corollary 6.3].

Corollary 2. *Suppose $\mathfrak{p}$ is a prime ideal in R such that dim $R_\mathfrak{p} = 1$ and $hd\ R/\mathfrak{p} \leq 2$. Then $\mathfrak{p}$ is a principal ideal.*

Proof: Since $hd_R R/\mathfrak{p} \geq hd_{R_\mathfrak{p}} R_\mathfrak{p}/\mathfrak{p}R_\mathfrak{p} = gl.\ \dim R_\mathfrak{p}$, it follows that the $gl.\ \dim R\mathfrak{p}$ is finite. (See reference 1, 1.6 and reference 3; VIII, 2.6[1].) Therefore $R_\mathfrak{p}$ is a regular local ring of dimension 1 (see reference 1, 1.9). Let $x_1, \ldots, x_t$ be a min. generating set for $\mathfrak{p}$. Then the $x_1, \ldots, x_t$ considered as elements in $R_\mathfrak{p}$ generate $\mathfrak{p}R_\mathfrak{p}$. Since $R_\mathfrak{p}$ is a regular local ring of dimension 1, we have that $\mathfrak{p}R_\mathfrak{p} = x_j R_\mathfrak{p}$ for some j. Let $(x_j) = \mathfrak{q}_1 \cap \ldots \cap \mathfrak{q}_l$ be a normal, primary decomposition for (x_j). From $x_j R_\mathfrak{p} = \mathfrak{p}R_\mathfrak{p}$ it follows that one of the $\mathfrak{q}_i$ is $\mathfrak{p}$. Let us say $\mathfrak{q}_1 = \mathfrak{p}$. Then for

y in $(\mathfrak{q}_2 \cap \ldots \cap \mathfrak{q}_t) - \mathfrak{p}$ we have that (x_j): $y = \mathfrak{p}$. Since x_j is not in $\mathfrak{M}\mathfrak{p}$ and *hd* $\mathfrak{p} \leq 1$, it follows from the previous proposition that $\mathfrak{p} = (x_j)$.

THEOREM 3. *Let R be a local domain of dimension ≤ 3 such that hd $R/\mathfrak{p} < \infty$ for all minimal prime ideals $\mathfrak{p}$. Then R is a unique factorization domain.*

Proof: Since R is a noetherian domain, it follows from reference 4; Lemma 1, pg. 408, that it suffices to show that each minimal prime ideal is principal in order to show that R is a unique factorization domain. But by Corollary 2, it will follow that a minimal prime ideal $\mathfrak{p}$ is principal if we can show that *hd* $R/p \leq 2$. Since *hd* $R/\mathfrak{p} < \infty$ we have by reference 1; 3.7 and 1.3 that *hd* $R/\mathfrak{p}$ + Codim $R/\mathfrak{p}$ = Codim $R \leq \dim R$. But Codim $R/\mathfrak{p} \geq 1$ and $\dim R \leq 3$. Thus *hd* $R/\mathfrak{p} \leq 2$, which completes the proof.

Since every module has finite homological dimension over a regular local ring, we have established

COROLLARY 4. *Every regular local ring of dimension ≤ 3 is a unique factorization domain.*

THEOREM 5. *Every regular local ring is a unique factorization domain.*

* Prior to this result, Zariski proved that if every complete regular local ring of dimension 3 is a unique factorization domain, then every complete regular local ring is a unique factorization domain (unpublished). Combining this with Mori's and Krull's result that a local ring is a unique factorization domain if it's completion is a unique factorization domain, we obtain another proof of this reduction theorem.

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*ON THE FALSITY OF EULER'S CONJECTURE ABOUT THE NON-EXISTENCE OF TWO ORTHOGONAL LATIN SQUARES OF ORDER $4t + 2$**

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1. Introduction.—The purpose of this paper is to prove a general theorem on the existence of pairwise orthogonal Latin squares (p.o.l.s.) of a given order and to give a counter example to Euler's conjecture[3] that there do not exist two p.o.l.s. of order $4t + 2$.

2. Definitions.—An arrangement of v objects (called treatments) in b sets (called blocks) will be called a pairwise balanced design of index unity and type $(v;\ k_1, k_2, \ldots, k_m)$ if each block contains either $k_1, k_2, \ldots,$ or k_m treatments which are all distinct $(k_i \leq v, k_i \neq k_j)$, and every pair of distinct treatments occurs exactly in one block of the design. If the number of blocks containing k_i treatments is b_i, then clearly

$$b = \sum_{i=1}^{m} b_i, \qquad v(v-1) = \sum_{i=1}^{m} b_i k_i (k_i - 1) \tag{1}$$

LEMMA 1. *Suppose there exists a set Σ of $q-1$ p.o.l.s. of order k, then we can construct a $q \times k(k-1)$ matrix P, whose elements are the symbols $1, 2, \ldots, k$ and such that any ordered pair $\binom{i}{j} i \neq j$, occurs as a column exactly once in any two-rowed submatrix of P.*

We can take the set Σ in the standard form in which the first row of each Latin square contains the symbols $1, 2, \ldots, k$ in that order. We then prefix to the set Σ a $k \times k$ square containing the symbol i in each position in the ith column. If we then write the elements of each square in a single row such that the symbol in the ith row and jth column occupies the nth position in the row, where $n = k(i-1) + j$ then we can display these squares (as in reference 2) in the form of an orthogonal array $A[k^2, q, k, 2]$ of q rows. By deleting the first k columns, we get the matrix P with the required properties.

Let γ be a column of k distinct treatments $t_1, t_2, \ldots, t_k$ in that order, then we shall denote by $P(\gamma)$, the $q \times k(k-1)$ matrix obtained by replacing the symbol i in P, by the treatment t_i occupying the ith position in $\gamma (i = 1, 2, \ldots, k)$. Clearly every treatment occurs exactly $k-1$ times in every row of $P(\gamma)$, and any ordered pair $\binom{t_i}{t_j}$ occurs as a column exactly once in any two-rowed submatrix of $P(\gamma)$.

THEOREM 1. *Let there exist a pairwise balanced design of index unity and type $(v; k_1, k_2, \ldots, k_m)$ and suppose there exist $q_i - 1$ p.o.l.s. of order k_i. If*

$$q = min\ (q_1, q_2, \ldots, q_m)$$

then there exist $q-2$ p.o.l.s. of order v.

Let the treatments of the design be $t_1, t_2, \ldots, t_v$, and let the blocks of the design (written out as columns) which contain k_i treatments be denoted by $\gamma_{i1}, \gamma_{i2}, \ldots, \gamma_{ib_i}$. Let P_i be the matrix of order $q_i \times k_i(k_i - 1)$ defined in Lemma 1, the elements of P_i being the symbols $1, 2, \ldots, k_i$. Let $C_{ij} = P_i(\gamma_{ij})$ be the matrix obtained from P_i and γ_{ij}. Retain only q rows of C_{ij} to get C_{ij}^*. From (1) the matrix

$$C^* = [C_{11}^*, C_{12}^*, \ldots, C_{1b_1}^*, \ldots, C_{i1}^*, C_{i2}^*, \ldots, C_{ib_i}^*, \ldots, C_{m1}^*, C_{m2}^*, \ldots, C_{mb_m}^*]$$

is of order $q \times v(v-1)$, and is such that any ordered pair of treatments $\binom{t_i}{t_j}$, $i \neq j$ occurs as a column exactly once in any two-rowed submatrix of C^*. Let C_0^* be a $q \times v$ matrix whose ith column contains t_i in every position $(i = 1, 2, \ldots, v)$. Then (from reference 2), the matrix $[C_0^*, C^*]$ is an orthogonal array $A[v^2, q, v, 2]$. Using two rows to coordinatize we get a set of $q-2$ p.o.l.s. of order v.

3. Counter Examples to Euler's Conjecture.—Consider the balanced incomplete block (BIB) design with parameters $v^* = 15$, $b^* = 35$, $r^* = 7$, $k^* = 3$, $\lambda^* = 1$. A resolvable solution is given in Table III of reference 1. To each block of the ith complete replication add a new treatment θ_i $(i = 1, 2, \ldots, 7)$ and take a new block consisting of the treatments $\theta_1, \theta_2, \theta_3, \theta_4, \theta_5, \theta_6, \theta_7$. We then get a pairwise balanced design of index unity and type $(22; 4, 7)$. Since there exist 3 p.o.l.s. of order 4, and 6 p.o.l.s. of order 7, it follows from the theorem that there exist two orthogonal Latin squares of order 22. The squares follow.

A detailed paper generalizing and improving the results of Mann[4] and Parker[5]

is being prepared where, among other things, it will be shown that there are an infinity of values of t for which there exist two or more p.o.l.s. of order $4t + 2$.

(L_1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	4	7	16	6	20	22	15	19	21	12	18	10	9	17	2	8	11	14	5	13	3
2	16	2	5	1	17	7	21	10	15	20	22	13	19	11	18	4	3	9	12	8	6	14
3	22	17	3	6	2	18	1	12	11	15	21	16	14	20	19	8	5	4	10	13	9	7
4	2	16	18	4	7	3	19	21	13	12	15	22	17	8	20	1	9	6	5	11	14	10
5	20	3	17	19	5	1	4	9	22	14	13	15	16	18	21	11	2	10	7	6	12	8
6	5	21	4	18	20	6	2	19	10	16	8	14	15	17	22	9	12	3	11	1	7	13
7	3	6	22	5	19	21	7	18	20	11	17	9	8	15	16	14	10	13	4	12	2	1
8	17	20	16	14	22	11	13	8	5	2	19	3	18	21	1	12	15	7	6	10	4	9
9	14	18	21	17	8	16	12	22	9	6	3	20	4	19	2	10	13	15	1	7	11	5
10	13	8	19	22	18	9	17	20	16	10	7	4	21	5	3	6	11	14	15	2	1	12
11	18	14	9	20	16	19	10	6	21	17	11	1	5	22	4	13	7	12	8	15	3	2
12	11	19	8	10	21	17	20	16	7	22	18	12	2	6	5	3	14	1	13	9	15	4
13	21	12	20	9	11	22	18	7	17	1	16	19	13	3	6	5	4	8	2	14	10	15
14	19	22	13	21	10	12	16	4	1	18	2	17	20	14	7	15	6	5	9	3	8	11
15	8	9	10	11	12	13	14	17	18	19	20	21	22	16	15	7	1	2	3	4	5	6
16	4	1	12	2	13	10	15	3	6	9	5	8	11	7	14	16	18	20	22	17	19	21
17	15	5	2	13	3	14	11	1	4	7	10	6	9	12	8	22	17	19	21	16	18	20
18	12	15	6	3	14	4	8	13	2	5	1	11	7	10	9	21	16	18	20	22	17	19
19	9	13	15	7	4	8	5	11	14	3	6	2	12	1	10	20	22	17	19	21	16	18
20	6	10	14	15	1	5	9	2	12	8	4	7	3	13	11	19	21	16	18	20	22	17
21	10	7	11	8	15	2	6	14	3	13	9	5	1	4	12	18	20	22	17	19	21	16
22	7	11	1	12	9	15	3	5	8	4	14	10	6	2	13	17	19	21	16	18	20	22

(L_2)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	16	22	2	20	5	3	17	14	13	18	11	21	19	8	4	15	12	9	6	10	7
2	4	2	17	16	3	21	6	20	18	8	14	19	12	22	9	1	5	15	13	10	7	11
3	7	5	3	18	17	4	22	16	21	19	9	8	20	13	10	12	2	6	15	14	11	1
4	16	1	6	4	19	18	5	14	17	22	20	10	9	21	11	2	13	3	7	15	8	12
5	6	17	2	7	5	20	19	22	8	18	16	21	11	10	12	13	3	14	4	1	15	9
6	20	7	18	3	1	6	21	11	16	9	19	17	22	12	13	10	14	4	8	5	2	15
7	22	21	1	19	4	2	7	13	12	17	10	20	18	16	14	15	11	8	5	9	6	3
8	15	10	12	21	9	19	18	8	22	20	6	16	7	4	17	3	1	13	11	2	14	5
9	19	15	11	13	22	10	20	5	9	16	21	7	17	1	18	6	4	2	14	12	3	8
10	21	20	15	12	14	16	11	2	6	10	17	22	1	18	19	9	7	5	3	8	13	4
11	12	22	21	15	13	8	17	19	3	7	11	18	16	2	20	5	10	1	6	4	9	14
12	18	13	16	22	15	14	9	3	20	4	1	12	19	17	21	8	6	11	2	7	5	10
13	10	19	14	17	16	15	8	18	4	21	5	2	13	20	22	11	9	7	12	3	1	6
14	9	11	20	8	18	17	15	21	19	5	22	6	3	14	16	7	12	10	1	13	4	2
15	17	18	19	20	21	22	16	1	2	3	4	5	6	7	15	14	8	9	10	11	12	13
16	2	4	8	1	11	9	14	12	10	6	13	3	5	15	7	16	20	17	21	18	22	19
17	8	3	5	9	2	12	10	15	13	11	7	14	4	6	1	20	17	21	18	22	19	16
18	11	9	4	6	10	3	13	7	15	14	12	1	8	5	2	17	21	18	22	19	16	20
19	14	12	10	5	7	11	4	6	1	15	8	13	2	9	3	21	18	22	19	16	20	17
20	5	8	13	11	6	1	12	10	7	2	15	9	14	3	4	18	22	19	16	20	17	21
21	13	6	9	14	12	7	2	4	11	1	3	15	10	8	5	22	19	16	20	17	21	18
22	3	14	7	10	8	13	1	9	5	12	2	4	15	11	6	19	16	20	17	21	18	22

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WHICH LIE GROUPS ARE HOMOTOPY-ABELIAN?

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1. Let H be a subgroup of a topological group G. We describe H as *homotopy-abelian* in G if $f \simeq \bar{f}: H \times H \to G$ where $f, \bar{f}$ are the maps given by

$$f(x, y) = xy = \bar{f}(y, x) \qquad (x, y \in H).$$

This is the case, for example, when G is pathwise-connected and H is conjugate to a subgroup whose elements commute with those of H. If H is homotopy-abelian in G, then any subgroup of H is homotopy-abelian in any group which contains G as a subgroup. A homotopy-abelian group is one which is homotopy-abelian in itself. There exist homotopy-abelian groups, such as the stable classical groups,[1] which are not abelian.

Consider the nonabelian classical groups[2]

$$SO(n) \quad (n \geqq 3), \qquad U(n) \quad (n \geqq 2), \qquad Sp(n) \quad (n \geqq 1);$$

subject to the standard embeddings $(m > n)$:

$$SO(n) \subset SO(m), \qquad U(n) \subset U(m), \qquad Sp(n) \subset Sp(m).$$

Because their elements commute with those of appropriate conjugate subgroups it follows that $SO(n)$, $U(n)$, $Sp(n)$ are homotopy-abelian in $SO(2n)$, $U(2n)$, $Sp(2n)$, respectively. We shall prove

THEOREM (1.1). *$U(n)$ is not homotopy-abelian in $U(2n - 1)$.*

THEOREM (1.2). *$Sp(n)$ is not homotopy-abelian in $Sp(2n - 1)$.*

The analogous statement is not true in the case of rotation groups since, for example, $SO(4)$ is homotopy-abelian in[3] $SO(7)$. However, we shall prove

THEOREM (1.3). *If n is odd, $SO(n)$ is not homotopy-abelian in $SO(2n - 2)$.*

COROLLARY (1.4). *If n is even, $SO(n)$ is not homotopy-abelian in $SO(2n - 4)$.*

These results imply that none of the groups $SO(n)$, $U(n)$, $Sp(n)$ is homotopy-abelian. Furthermore we shall prove

THEOREM (1.5). *The classical structure classes contain no Lie group which is homotopy-abelian but not abelian.*

Although it seems probable that the only homotopy-abelian Lie groups are abelian, we have not been able to eliminate the possibility that certain of the exceptional groups are homotopy-abelian. Our method is a development of one employed by Samelson[4] to show that $Sp(1)$ cannot be homotopy-abelian. It involves consideration of the pairing in the homotopy groups of G, called the Samelson product, whose definition[5] depends on the operation of commutation in G. This pairing is related to the Whitehead product in the homotopy groups of

the classifying space of G, which can be studied by techniques of cohomology. Details are as follows.

2. Take cohomology with coefficients in Z_p, the ring of integers modulo p, where p is an odd prime. Let B be a classifying space for the topological group G. Suppose that $H^*(B)$ is a polynomial algebra on even-dimensional generators x_1, $\ldots$, x_q. This will be the case, for example, when G is a compact connected Lie group with no p-torsion.[6] An element $x \in H^*(B)$ can be expressed uniquely in the form $p_1 + p_2 + \ldots + p_m + \ldots$, where p_m is a homogeneous polynomial of degree m in the generators. We say that x is *decomposable* when $p_1 = 0$, and then we refer to p_2 as the *quadratic part* of x. We say that x is *dependent* if it can be obtained from an element of lesser dimension by application of one of the stable cohomology operations.

Suppose, given an element $\alpha \in \pi_r(B)$, where $r \geqq 1$. We identify $H^r(S^r)$ with Z_p. Define $a_i \in Z_p$, where $1 \leqq i \leqq q$, to be $\alpha^* x_i$ if $\dim x_i = r$, to be zero otherwise. We refer to a_i as the *index* of x_i, with regard to α, and describe a decomposable element $x \in H^{2r}(B)$ as *significant*, with regard to α, if $\Sigma g_{jk} a_j a_k \neq 0$, where $\Sigma g_{jk} x_j x_k$ is the quadratic part of x.

LEMMA (2.1). *Suppose that the order of the Whitehead product* $[\alpha, \alpha] \in \pi_{2r-1}(B)$ *is finite and prime to* p. *Then no dependent element of* $H^{2r}(B)$ *can be significant, with regard to* α.

Let A be the space obtained from $S^r \times S^r$ by identifying $(x, e) = x = (e, x)$ $(x \in S^r)$, where $e \in S^r$ is the basepoint. The complement of S^r in A is a $2r$-cell, attached by the Whitehead product of the identity on S^r with itself. Write $m\alpha = \alpha'$, where m is the order of $[\alpha, \alpha]$. Since $[\alpha', \alpha'] = 0$, there exists a map $u: A \to B$ such that $u \mid S^r$ represents α'. Consider the induced homomorphism $u^*: H^*(B) \to H^*(A)$, which is trivial except in dimensions 0, r, $2r$. If $x_i \in H^r(B)$ then $u^* x_i = a_i' z = m a_i z$, where z generates $H^r(A)$ and a_i' denotes the index of x_i with regard to α'. Let $x \in H^{2r}(B)$ be decomposable. Then

$$u^* x = u^* p_2 + u^* p_3 + \ldots = u^* p_2 = c z^2,$$

where $c = \Sigma g_{jk} a_j' a_k' = m^2 \Sigma g_{jk} a_j a_k$. If $a_i \neq 0$ for some value of i then $r = \dim x_i$, which is even, and so $z^2 \neq 0$. Hence, and since m is prime to p, it follows that $u^* x \neq 0$ when x is significant. Let x be dependent, as well as decomposable. Then $u^* x = 0$, by naturality and stability of the cohomology operation, since the suspension of S^r is a retract of the suspension of A. Therefore x is not significant, which proves (2.1).

Since B is the base space of a principal G-bundle whose total space is contractible, there is an isomorphism $\Delta: \pi_m(B) \approx \pi_{m-1}(G)$ in the homotopy sequence of the fibration. Write $\Delta\alpha = \beta$. Let $y_1, \ldots y_q$ denote the elements of $H^*(G)$ which correspond under transgression to $x_1, \ldots, x_q$, respectively. Suspension identifies $H^{r-1}(S^{r-1})$ with $H^r(S^r)$, and hence with Z_p. If $\dim y_i = r-1$ then $\beta^* y_i = a_i$, the index of x_i with regard to α. We shall therefore refer to a_i alternatively as the index of y_i with regard to β. Significance with regard to β is defined to mean the same as does significance with regard to α. The Samelson product $\langle \beta, \beta \rangle \in \pi_{2r-2}(G)$ coincides with[7] $\pm \Delta[\alpha, \alpha]$. Hence (2.1) leads immediately to

THEOREM (2.2). *Suppose that* $H^{2r}(B)$ *contains a dependent element which is*

significant, with regard to $\beta \in \pi_{r-1}(G)$. *Then the order of* $<\beta, \beta>$ *is either infinite or else divisible by* p.

We refer to $<\beta, \beta>$ as the *Samelson square* of β. When G is a Lie group the Samelson product always has finite order.[8]

3. For example take $G = U(q)$, whose homology is torsion-free. The generators of $H^*(B)$ are $x_1, \ldots, x_i, \ldots, x_q$, with dim $x_i = 2i$. We apply (2.2), with $r = q + 1$, to prove

THEOREM (3.1). *If* $q = 2i-1$ *and* $i < p \leqq q$ *then* $\pi_q(U(q))$ *contains an element whose Samelson square has order divisible by* p.

The values of the reduced power operations in $H^*(B)$ have been given by Borel and Serre.[9] Their results show that $x = -\mathcal{P}_p^1 x_{2i-p+1}$ is decomposable, with quadratic part

$$x_1 x_{2i-1} + \ldots + i x_i x_i + \ldots + (p-1) x_{p-1} x_{2i-p+1}.$$

Since p is regular for $U(q)$, in the sense of Serre,[10] it follows that x_i has nonzero index for some $\beta \in \pi_q(U(q))$, and so x is significant, as well as dependent. For the other classical groups we obtain similarly

THEOREM (3.2). *Let* $q = 2i - 1$, *where* i *is even, and let* $i < p \leqq q$. *Then* $\pi_q(Sp(i-1))$ *and* $\pi_q(SO(q))$ *each contain an element whose Samelson square has order divisible by* p.

It can also be shown by this method that the exceptional groups G_2 and F_4 admit nontrivial Samelson squares. We have not been able to include E_6, E_7 and E_8 because the appropriate information about cohomology is not yet available.

4. A straightforward consequence of the definition of the Samelson product is

LEMMA (4.1). *Let* H *be a subgroup which is homotopy-abelian in* G. *Then the Samelson square annihilates the image of the injection* $\pi_q(H) \to \pi_q(G)$.

We are now ready to deduce the results stated in §1. Recall (Bertrand's Postulate) that for $i \geqq 1$ there exists at least one prime p such that $i < p \leqq 2i$. In (3.1), the injection maps $\pi_q(U(i))$ onto $\pi_q(U(q))$, by exactness of fiber homotopy sequences; and so (1.1) follows from (3.1), with $i = n$, and (4.1). The deduction of (1.2) is similar, using the first part of (3.2) with $i = 2n$. To prove (1.3), let q and i be as in (3.2). Then[11] the kernel of the injection $\pi_{2q}(SO(q)) \to \pi_{2q}(SO(q+1))$ has order a power of 2. Moreover, by considering the homology[12] of the factor space $SO(q)/SO(i+1)$, we see that the cokernel of the injection $\pi_q(SO(i+1)) \to \pi_q(SO(q))$ also has order a power of 2. Thus, the second part of (3.2) implies that the image of the injection $\pi_q(SO(i+1)) \to \pi_q(SO(q+1))$ contains an element whose Samelson square has order divisible by p. Hence and from (4.1), we obtain (1.3) with $n = i + 1$. It is possible to carry these arguments somewhat further, and prove

THEOREM (4.2). $Sp(n)$ *is not homotopy-abelian in either* $U(4n-1)$ *or* $SO(4n)$.

THEOREM (4.3). *If* n *is odd,* $SO(n)$ *is not homotopy-abelian in* $U(2n-3)$.

The embeddings here are the standard ones, and of course $n \geqq 3$ in (4.3). There are many similar relations which call for investigation, but we have been unable to establish any further systematic results.

Finally we prove (1.5). Let G' be a 1-connected Lie group. If G is locally isomorphic to G' then there exists a covering homomorphism $G' \to G$ which induces an isomorphism of the higher homotopy groups, including the Samelson product.

Thus when one of the groups in a structure class has a nontrivial Samelson product (not involving the fundamental group) then so do all the others. Hence (1.5) follows from (3.1) and (3.2).

* Holder of a postdoctoral fellowship of the National Science Foundation.

[1] Bott, R., "The stable homotopy of the classical groups," these PROCEEDINGS, **43**, 933–5 (1957).

[2] The corresponding results for $O(n)$ and $SU(n)$ can easily be deduced by using the expressions $O(n) = SO(n) \times O(1)$, $U(n) = SU(n) \times U(1)$.

[3] Because $SO(4)$ is homotopy-abelian in $SO(8)$, which contains $SO(7)$ as a retract.

[4] Samelson, H., "Groups and spaces of loops," *Comm. Math. Helv.*, **28**, 278–87 (1954).

[5] Whitehead, G. W., "On mappings into group-like spaces," *Comm. Math. Helv.*, **28**, 320–27 (1954).

[6] Borel, A., "Sur la cohomologie des espaces fibrés...," *Ann. Math.*, **57**, 115–207 (1953); see (7.2) and (19.1).

[7] Barratt, M. G., I. M. James, and N. Stein, "Whitehead products and projective spaces," (to appear).

[8] James, I. M., "On Lie groups and their homotopy groups," *Proc. Cambridge Philosophical Soc.* (to appear).

[9] Borel, A., and J.-P. Serre, "Groupes de Lie et puissances réduites de Steenrod," *Am. J. Math.*, **75**, 409–48 (1953); see Chapter III.

[10] Serre, J.-P., "Groupes d'homotopie et classes de groupes abéliens," *Ann. Math.*, **58**, 258–94 (1953); see Chapter V.

[11] See reference 9, (18.2).

[12] See reference 6, (10.2).

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*A QUANTITATIVE FORMULATION OF SYLVESTER'S LAW OF INERTIA**

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1. Sylvester's law of inertia can be expressed in the following way. Let $H = (h_{\mu\nu})$ be an hermitian matrix of order n and S an arbitrary real nonsingular matrix of the same order. Denote the eigenvalues of H, ordered increasingly, by

$$\lambda_1 \leq \lambda_2 \leq \ldots \leq \lambda_n \tag{1}$$

and those of S^*HS by

$$\Lambda_1 \leq \Lambda_2 \leq \ldots \leq \Lambda_n. \tag{2}$$

Then the numbers of positive, negative and vanishing terms in (2) are the same as in (1).

This result can be sharpened in the following way:

THEOREM 1. *We have*

$$\Lambda_\nu = \theta_\nu \lambda_\nu \qquad (\nu = 1, 2, \ldots, n) \tag{3}$$

where θ_ν lie between the smallest eigenvalue p_1 and the largest eigenvalue p_n of the positive definite matrix S^*S.[1]

2. This result is rather unexpectedly precise. If we take for H the unity matrix, all λ_ν become 1 and Λ_ν are just the eigenvalues of S^*S. If S is unitary we have $p_1 = p_n = 1$, $\theta_\nu = 1$, which is just the invariance of eigenvalues under a unitary transformation. We see that the result of Theorem 1 is the best for any fixed S and the best for any fixed H.

3. By continuity one sees that the relation (3) of Theorem 1 remains true if S becomes singular. In this case p_1 becomes 0. From this follows in particular the following extension of Sylvester's law of inertia to *singular* transformations.

THEOREM 2. *If H is an hermitian matrix of order n and S an arbitrary real matrix of the same order, then the numbers of positive and negative eigenvalues of the matrix S^*HS do not exceed the corresponding numbers for the matrix H.*

4. Theorem 1 is easily deduced from a theorem which we proved recently.[2]

THEOREM 3. *Let H be an hermitian matrix of order n with the eigenvalues (1) and P a positive definite or semidefinite hermitian matrix of the same order, with the minimal eigenvalue p_1 and the maximal eigenvalue p_n. Then, if the eigenvalues of the product PH are denoted by (2) we have the relations (3) where the θ_ν lie between p_1 and p_n.*

Theorem 1 follows from Theorem 3 if we use

$$S(S^*HS)S^{-1} = PH, \qquad P = SS^*, \tag{4}$$

and observe that SS^* is a positive definite matrix which has the same eigenvalues as S^*S. On the other hand it is well known that a positive definite matrix can always be written in the form SS^*. Therefore Theorem 3 follows from Theorem 1 for the case of a definite P and is then easily extended by continuity to the case of a semidefinite P.

5. In what follows we give a very simple direct proof of Theorem 1. In this way we obtain by the argument of section 4 also a new proof of Theorem 3 considerably simpler than that given in the paper quoted above. We make use of

LEMMA 1. *If H and H_1 are two hermitian matrices of the order n, if the eigenvalues of H and H-H_1 ordered increasingly are respectively λ_ν, Λ_ν ($\nu = 1, \ldots, n$) and if the eigenvalues of H_1 are contained between u and v, $u \leq v$, then we have*

$$\Lambda_\nu = \lambda_\nu - h_\nu, \; u \leq h_\nu \leq v \qquad (\nu = 1, \ldots, n). \tag{5}$$

It is then sufficient to argue as follows. For any λ_k ($k = 1, \ldots, n$), $H - \lambda_k I$ has zero as its kth eigenvalue. Therefore, by Sylvester's law of inertia, the same is true for the matrix $S^*(H - \lambda_k I)S$. But by Lemma 1 the kth eigenvalue of this matrix, in virtue of

$$S^*(H - \lambda_k I)S = S^*HS - \lambda_k S^*S \tag{6}$$

can be written in the form $\Lambda_k - \lambda_k\theta_k$ where θ_k is contained between p_1 and p_n. Since this difference is zero we obtain the formula (3).

6. As to Lemma 1, it is contained in Theorem I of H. Weyl's paper quoted above (see p. 445 of that paper), if we take $m = 0$ in the formulas (1) and (2) of Weyl's Theorem I. On the other hand Lemma 1 follows very easily from E. Fischer's maximum-minimum principle. Indeed, by this principle we have

$$\Lambda_\nu = \max_{L_{n-\nu+1}} \min_{\substack{|\xi|=1 \\ \xi \prec L_{n-\nu+1}}} \xi^*(H - H_1)\xi = \max_{L_{n-\nu+1}} \min_{\substack{|\xi|=1 \\ \xi \prec L_{n-\nu+1}}} (\xi^*H\xi - \xi^*H_1\xi) \tag{7}$$

where Min is taken over all vectors ξ of length 1 contained in a linear manifold $L_{n-\nu+1}$ of dimension $n - \nu + 1$ and Max over all linear manifolds $L_{n-\nu+1}$. Since here $\xi^* H_1 \xi$ lies between u and v, (5) follows immediately.

7. In order to derive the results corresponding to (3) for the *moduli of the eigenvalues* we prove first

LEMMA 2. *Assume that two sets of n positive numbers,*

$$x_1, \ldots, x_n; y_1, \ldots, y_n \tag{8}$$

satisfy the inequalities

$$qx_\nu \leq y_\nu \leq Qx_\nu \qquad (\nu = 1, \ldots, n) \tag{9}$$

for $0 < q < Q$. Then, if both sequences (8) rearranged in increasing order become, respectively,

$$x_1' \leq x_2' \leq \ldots \leq x_n', \tag{10}$$

$$y_1' \leq y_2' \leq \ldots \leq y_n', \tag{11}$$

we have

$$qx_\nu' \leq y_\nu' \leq Qx_\nu' \qquad (\nu = 1, \ldots, n). \tag{12}$$

To prove this, observe that it is sufficient to prove the right-side inequalities in (12) since the left-side inequalities then follow if we interchange x_ν and y_ν and replace Q by $1/q$. Further, in proving the right-side inequalities in (12) it is sufficient to assume $Q = 1$, since we can always divide all y_ν by Q.

8. Thus Lemma 2 follows from

LEMMA 3. *If we have two sets of n numbers (8) where for each v*

$$x_\nu \leq y_\nu \qquad (\nu = 1, \ldots, n) \tag{13}$$

and if the x_ν and y_ν rearranged in increasing order give (10) and (11), then we have

$$x_k' \leq y_k' \qquad (k = 1, \ldots, n). \tag{14}$$ [3]

But Lemma 3 is almost obvious. There are by (11) at least k among the y_ν which are $\leq y_k'$. Therefore by (13) there are at least k among the x_ν which are $\leq y_k'$. And then by (10) we have certainly (14).

It is easy to see that Lemma 2 remains true if the x_ν and y_ν are assumed non-negative instead of positive. Further, if in all inequalities (9) the equality sign is excluded, the same is true of the inequalities (12).

9. We have from (3)

$$p_1 |\lambda_\nu| \leq |\Lambda_\nu| \leq p_n |\lambda_\nu| \qquad (\nu = 1, \ldots, n);$$

therefore using Lemma 2 we obtain

THEOREM 4. *If under assumptions of Theorem 1 the λ_ν and the Λ_ν are rearranged in the order of increasing moduli,*

$$|\lambda_1'| \leq |\lambda_2'| \leq \ldots \leq |\lambda_n'|, \ |\Lambda_1'| \leq |\Lambda_2'| \leq \ldots \leq |\Lambda_n'|, \tag{15}$$

then we have

$$\Lambda_\nu' = \theta_\nu \lambda_\nu', \ p_1 \leq \theta_\nu \leq p_n \qquad (\nu = 1, \ldots, n). \tag{16}$$

10. Theorem 4 can now be generalized, introducing the whole sequence of eigenvalues of S^*S:

$$p_1 \leq p_2 \leq \ldots \leq p_n. \tag{17}$$

We are going to prove

THEOREM 5. *If under the assumptions of Theorem* 1 *the eigenvalues* λ_ν' *of* H *and* Λ_ν' *of* S^*HS *are arranged in such a way that* (15) *holds, then we have for any* k *with* $1 \leq k \leq n$:

$$p_1 p_2 \ldots p_k \left|\lambda_1' \ldots \lambda_k'\right| \leq \left|\Lambda_1' \ldots \Lambda_k'\right| \leq p_{n-k+1} p_{n-k+2} \ldots p_n \left|\lambda_1' \ldots \lambda_k'\right|. \tag{18}$$

We prove this in the usual way, using compound matrices. We have indeed for kth compounds

$$(S^*HS)^{(k)} = (S^*)^{(k)} H^{(k)} S^{(k)}$$

and here the eigenvalues of $(S^*HS)^{(k)}$ are by the theorem of Rados all products of k factors Λ_ν' with different indices. The eigenvalues of $H^{(k)}$ are obtained in the same way from the λ_ν' while the maximal and minimal eigenvalues of $S^{*(k)}S^{(k)} = (S^*S)^{(k)}$ are $p_n \ldots p_{n-k+1}$ and $p_1 \ldots p_k$. Therefore Theorem 5 follows applying Theorem 4 to $H^{(k)}$ and $S^{(k)}$.

11. If we try to apply the method of compound matrices to the eigenvalues λ_ν and Λ_ν instead of to their moduli, the difficulty arises, that the order of the eigenvalues of $H^{(k)}$ for instance is not completely determined by the order of the λ_ν, as soon as the λ_ν assume different signs. However, if among the λ_ν there is only one negative, the smallest eigenvalue of $H^{(k)}$ is $\lambda_1, \ldots \lambda_k$ and the greatest $\lambda_n \ldots \lambda_{n-k+1}$. If exactly two among the λ_ν are negative, then the smallest eigenvalue of $H^{(k)}$ is for $k < n$ certainly $\lambda_1\lambda_3\lambda_4 \ldots \lambda_{k+1}$. We obtain therefore

THEOREM 6. *If under the assumptions of Theorem 1 we have* $\lambda_1 < 0 < \lambda_2$, *then for* $1 \leq k \leq n$

$$\Lambda_1 \ldots \Lambda_k = \theta\lambda_1 \ldots \lambda_k,\ \Lambda_{n-k+1} \ldots \Lambda_n = \theta'\lambda_{n-k+1} \ldots \lambda_n, \tag{19}$$

where θ *and* θ' *lie between* $p_1 \ldots p_k$ *and* $p_{n-k+1} \ldots p_n$. *If we have* $\lambda_1 \leq \lambda_2 < 0 < \lambda_3$, *then for* $2 \leq k < n$

$$\Lambda_1\Lambda_3\Lambda_4 \ldots \Lambda_{k+1} = \theta\lambda_1\lambda_3\lambda_4 - \lambda_{k+1}, \tag{20}$$

where θ *lies between* $p_1 \ldots p_k$ *and* $p_{n-k+1} \ldots p_n$.

12. We obviously obtain results analogous to (19) and (20) if we assume that exactly one or exactly two of the λ_ν are positive.

By the argument of section 4 it follows that Theorems 4 and 5 remain valid under the hypotheses of Theorem 3. Further the identity

$$HP - \lambda I = P(H - \lambda P_1) \qquad PP_1 = I,$$

allows us to obtain at once results completely analogous to those of Theorems 4 and 5 for eigenvalues of $H - \lambda P_1$, for a positive P_1, as compared with the eigenvalues of H.

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[1] In the special case where S is a *diagonal matrix* a result corresponding to (3) in the theory of integral equations is, in principle, contained in *H. Weyl*, "Das asymptotische Verteilungsgesetz der Eigenwerte der linearen partiellen Differentialgleichungen (mit einer Anwendung auf die Theorie der Hohlraumstrahlung)," *Math. Ann.*, **71**, 441–479 (1912); see Weyl's theorem IV, p. 447.

[3] Ostrowski, A., "Uber Eigenwerte von Produkten Hermitenscher Matrizen," *Hamburger Math. Abh.* **23**, 60–68 (1959).

[2] For a statement equivalent to that of Lemma 3 see G. Pólya and M. Schiffer, "Convexity of functionals by transplantation," *Journal d'analyse mathématique*, **3**, 280 (1953/4).

*ENERGY ABSORPTION BY CHARGE CARRIERS OF NEGATIVE EFFECTIVE MASS IN CRYSTALS**

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The energy bands of crystals always have regions in wavevector space in which the effective mass of the carrier is negative in one or more orthogonal directions. Such bands were discovered by Dresselhaus, Kip, and Kittel.[1] In certain circumstances it is possible to conduct experiments such as cyclotron resonance and Faraday rotation to exhibit the negative-mass carriers, and it is therefore of interest to consider their properties. The statements one can make in the limit of the effective mass approximation include the following:

(*a*) Negative-mass carriers are accelerated by electric, magnetic, and gravitational fields in a direction opposite to positive-mass carriers of the same charge type. Thus, for example, a cyclotron resonance experiment on germanium or silicon with the aid of circularly-polarized radiation can discriminate between the positive- and negative-mass regions of the band of heavy holes: the sense of polarization for which resonance occurs will be opposite for the positive- and negative-mass regions.

(*b*) A system of carriers all of negative mass cannot exist in thermal equilibrium.

(*c*) A distribution of negative- and positive-mass carriers in thermal equilibrium will absorb (and not emit) energy from an electromagnetic field. That is, the electrical resistivity is always positive in equilibrium. The use of the negative resistance characteristic of negative-mass regions of **k**-space to construct amplifiers, as proposed by Kroemer,[2] will demand, as he recognizes, special nonequilibrium carrier distributions maintained by auxiliary power sources.

Consider now a group of carriers having negative mass $-|m|$. We assume for the present that a positive relaxation time τ may be defined in the usual way. We would expect the equation of motion for the drift velocity $\mathbf{v}$ in the constant electric field $\mathcal{E}$ to be

$$-|m|\left(\frac{d\underset{\sim}{v}}{dt}+\frac{1}{\tau}\underset{\sim}{v}\right)=e\mathcal{E}, \tag{1}$$

which has the steady-state solution $\mathbf{v} = -e\mathcal{E}\tau/|m|$. The electrical conductivity is

$$\sigma = -Ne^2\tau/|m| \tag{2}$$

and is negative. This result suggests that a semiconductor element would show negative resistance as long as, for equal $\tau/|m|$, there were more negative- than positive-mass carriers; an even stronger consequence of this result is that cyclotron resonance with circular polarization at the position of the negative-mass resonance should be characterized by emission rather than absorption.

Both of these statements offer the possibility of flagrant violations of the laws of thermodynamics. Consider the Planck statement of the Second Law: It is impossible to construct a system operating in a complete cycle which produces no effect except the exchange of heat at a reservoir at one positive temperature and the output of mechanical (or electrical) work. This principle can obviously be violated by a negative resistance element connected to a pair of condenser plates: positive and negative charges are separated by the element, and we can use the electric field between the plates to do work on conventional devices.

To show *how* the Second Law is preserved we have to establish that a system of negative and positive masses in thermal equilibrium will have positive resistance. It is true that a system of carriers all having negative mass will have negative resistance, but it is easily seen that *a system of carriers all having negative mass cannot exist in thermal equilibrium with a heat bath at a positive temperature.* If $\partial^2E/\partial k^2$ is everywhere negative the energy is unbounded below and thus a condition of thermal equilibrium cannot be established for any positive temperature. Further, for such an energy surface the entropy will increase monotonically as the energy decreases; thus

$$\frac{\partial S}{\partial U} = \frac{1}{T} \tag{3}$$

is negative. However, actual energy surfaces in crystals obviously cannot have $\partial^2E/\partial k^2$ everywhere negative, for the surfaces are bounded below and have both positive- and negative-mass regions.

We consider as an illustration the simplified problem of the electrical resistivity of a one-dimensional solid having a conduction band $E(k)$ with regions of both positive and negative effective masses.[3] Let $f_0(k)$ be the distribution function in thermal equilibrium; that is, the probability that the state k be occupied. In the electric field $\mathcal{E}$ the first-order solution of the transport equation is

$$f = f_0 - e\mathcal{E}\tau\frac{\partial f_0}{\partial k}, \tag{4}$$

where τ is the relaxation time and may be a function of k. We suppose that τ is always positive, as otherwise thermal equilibrium could never be established. The electric current density is

$$\begin{aligned} J &= \int eufdk = \int e(dE/dk)fdk \\ &= -e^2\mathcal{E}\int \tau(dE/dk)(\partial f_0/\partial k)dk, \end{aligned} \tag{5}$$

using (4) and noting that $\int f_0(\partial E/\partial k)dk$ vanishes. Now

$$\frac{dE}{dk}\frac{\partial f_0}{\partial k} = \frac{\partial f_0}{\partial E}\left(\frac{dE}{dk}\right)^2, \tag{6}$$

so that the sign of the integrand in (11) is completely determined by the sign of $\partial f_0/\partial E$. But the equilibrium distribution function f_0 is always a monotonically decreasing function of the energy; thus $\partial f_0/\partial E$ is always negative and the current is therefore always in the direction of the electric field $\mathcal{E}$. The resistance is positive.

These statements apply to any passive circuit element. The restriction to one dimension is easily lifted, but nothing new emerges from the discussion in three dimensions. The result depends in no way on the shape $E(k)$ of the band. The derivation applies only to the Ohmic term in the resistance, but the earlier thermodynamic argument is of general validity. Mathematically our result follows because $\partial f_0/\partial k$ is always opposite in sign to $\partial E/\partial k$, and thus the sign of the integrand in (6) is completely determined, regardless of the shape of the band. For cyclotron resonance one can see easily by a detailed balance argument that the absorption will be positive, regardless of the sign of the mass. In thermal equilibrium the population of a lower state is always greater than the population of an upper state. As the transition rates between any two states must be equal in an external rf field, there must be a net upward flux of particles, corresponding to absorption.

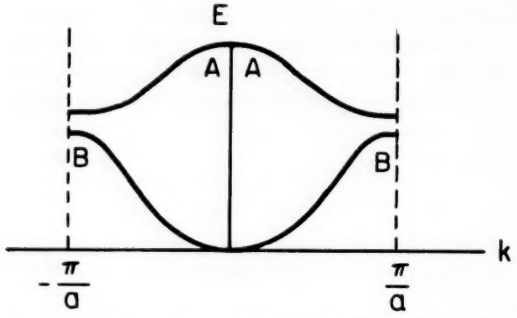

FIG. 1.—Negative mass regions of one-dimensional energy surface at AA and BB.

It is, however, entirely possible to obtain a negative resistance from an active circuit element in which the carriers are maintained in a steady excited nonequilibrium distribution. To obtain negative resistance from a general nonequilibrium distribution $g(E)$, we must have $\partial g/\partial E$ positive for some region of k-space *and* some negative mass states must be occupied. Such a distribution might be maintained by photoelectric ionization of donors or acceptors along with the action of some recombination mechanism. To obtain negative resistance, the injection process should favor the creation of carriers in negative-mass regions and the recombination processes should be sufficiently rapid to keep the carriers from being scattered out of the negative-mass regions.

It suffices for the sake of a very special example to neglect entirely the thermal processes; we consider a dielectric crystal at 0°K with electrons injected at $\pm k_0$ by optical excitation. In Figure 1, we want the injection to occur at a point such as A. In the absence of an electric field the distribution function near point A might satisfy an equation of the form

$$\frac{\partial f}{\partial t} = \alpha \frac{\partial f}{\partial E} - \frac{1}{S} f, \qquad (\alpha, S = \text{constants}) \tag{7}$$

where α is assumed to be constant and the term in $\partial f/\partial E$ simulates the effect of energy loss by phonon excitation and the term $-f/S$ simulates recombination. Both terms are highly schematic and are only illustrative. In the presence of an

electric field the steady-state transport equation becomes

$$e\mathcal{E}\frac{df}{dk} = \alpha\frac{\partial f}{\partial E} - \frac{1}{S}f. \tag{8}$$

For $\mathcal{E} = 0$ the solution is

$$\begin{aligned} f_0 &\propto e^{E/S\alpha} \text{ for } E < E(A) \\ &= 0, \qquad E > E(A). \end{aligned} \tag{9}$$

Thus on this special model the unperturbed distribution of injected carriers simulates a negative temperature distribution and it becomes possible in principle to have a negative resistance element, if the relevant masses are principally negative.

This note was prepared during the 1958 Solid State Summer School at the École Normale Supérieure in Paris. I am grateful to Professor P. Aigrain for his kind hospitality.

* Assisted by the National Science Foundation.

[1] Dresselhaus, Kip, and Kittel, *Phys. Rev.*, **98,** 368 (1955).

[2] Kroemer, H., *Phys. Rev.*, **109,** 1856 (1958); also private communication.

[3] Similar considerations have been carried out independently by Dr. Peter Kaus of the University of Southern California. Dr. S. Rodriguez of the University of Washington has successfully extended our argument to motion in three dimensions in a magnetic field. I am grateful to both workers for the private communication of their results. Cyclotron resonance experiments bearing on the negative mass regions have been carried out by Dousmanis and coworkers.

SEX INDUCED WITH ECDYSONE

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Introduction.—This paper deals with the induction of sex in the protozoan flagellates of the wood-feeding roach *Cryptocercus punctulatus*. Except for a few days following hatching, the hind-gut of all individuals of this insect is always filled to its utmost capacity by these symbiotic protozoa without which the roach cannot live.[1] These parasitic flagellates, many of which are unusually large cells, represent a great diversity of structure, comprising 9 families, 14 genera, and over 30 species.

However, their diversity in structure, great as it is, is surpassed by the number of very different types of sexual cycles these organisms undergo.[2]

Sex in them occurs only during the molting period of their insect host. Except in the first three instars, there is usually only one molting period a year; sex never occurs in the protozoa of adults and intermolt nymphs. Any procedure that prolongs the molting period affects sex in the same way; and any procedure that prevents molting, prevents sex.

Some genera of these flagellates have sexual processes that are as well developed as those of higher organisms; that is, they have highly differentiated types of gametogenesis, fertilization, and meiosis. In other genera, sex is less well developed,

and in some it is simple. However, all of these types are induced, as the experiments described here prove, by the molting hormone ecdysone.

Under natural conditions some of the genera of flagellates begin their sexual cycles much earlier in the molting period than others.[3] As shown in Table 1, *Barbulanympha*, *Saccinobaculus*, and *Oxymonas* begin their sexual cycles 40–50 days before their host undergoes ecdysis, while *Trichonympha* does not begin its sexual cycle until 5–6 days before ecdysis; yet, except for three species, they all complete their cycles at the same time. In all of these cycles, gametogenesis is the first sexual process. In the haploid genera, gametogenesis and meiosis are separate processes, but in diploids they occur at the same time. Thus, in haploids the sexual cycle ends with meiosis, while in diploids it ends with fertilization.

The data shown in Table 1 were obtained by isolating over 500 individual roaches whose protozoa were beginning a certain stage in their sexual cycles. Each roach was observed many times daily and the time of its ecdysis noted. The data after ecdysis were obtained by killing roaches at frequent intervals and noting the stages of development of their protozoa. By such a procedure an accurate timetable of normal events in sexuality of the different genera of protozoa becomes available for comparison with what occurs under experimental conditions. This table enables one to compare the sexual behavior, in time, under natural conditions with that which is produced by the injection of different amounts of the insect hormone ecdysone. This hormone has been variously termed, growth and differentiation hormone, molting hormone, and prothoracic gland hormone.

There is general agreement among insect endocrinologists that ecdysone, extracted by Butenandt and Karlson,[4] is produced by the prothoracic glands of insects. The corpus allatum produces the juvenile hormone. The hormone produced by the neurosecretory cells of the insect brain serves to activate the prothoracic glands. When the neurosecretory cells are removed, the prothoracic glands do not continue the production of ecdysone, and gametogenesis in *Trichonympha*, the genus studied most thoroughly, stops in less than 2 hours, followed by degeneration within 6–10 hours.[5] This means the prothoracic glands must be continuously activated by the brain hormone if the development of the sexual cycles is to continue. Sex in these flagellates, then, is indirectly dependent on the neurosecretory cells of their host.

Ecdysone is measured in Calliphora units, one unit being 0.0075 μg crystalline α-ecdysone. In the experiments reported here purified amorphous hormone concentrate was used. It was dissolved in insect Ringer and injected either into the thorax or the abdominal cavity of the roach by means of a special syringe which measured accurately amounts of $^1/_{10,000}$ ml. Both sites of injection gave the same results.

A total of 67 experiments was carried out. Adults and all nymphal instars except the first two were inoculated with ecdysone. In most of the experiments, 4th and 5th instar nymphs were used. The detailed results of only certain experiments are reported in this paper. A further report will be given later elsewhere.

Experiments.—In the first series of experiments 4th and 5th instar nymphs, in which *Barbulanympha*, *Saccinobaculus*, and *Oxymonas* had just begun gametogenesis, were each injected with 100 units of ecdysone. Ecdysis occurred 7–8 days later, instead of 42–47 days later as would have been the case under natural con-

ditions (Table 1). When 500 units were administered under these same conditions, ecdysis occurred after 6–7 days. In both of these experiments (100 and 500 units) the sexual cycles of the protozoa in every roach used were in exactly the same stage of development at ecdysis as under natural conditions. In other words, when a roach which would normally undergo ecdysis in 45 days is made to do so in 7–8 days the sexual cycles of its protozoa are affected in no discernible way other than time. The activities of the protozoa are closely tuned to those of their host.[6]

When only 50 units of ecdysone are given under the same conditions as were 100 and 500, ecdysis takes place on the natural schedule, i.e., 42–47 days later and all the sexual cycles of all the genera of protozoa save one, *Barbulanympha*, are also on their normal, natural schedule. This experiment, like others not reported here, shows that *Barbulanympha* is more sensitive to ecdysone than the other genera. It completes the sexual cycle 7–8 days after ecdysone is injected into its host. But a further reduction in ecdysone to 25 units will not induce sex in this genus.

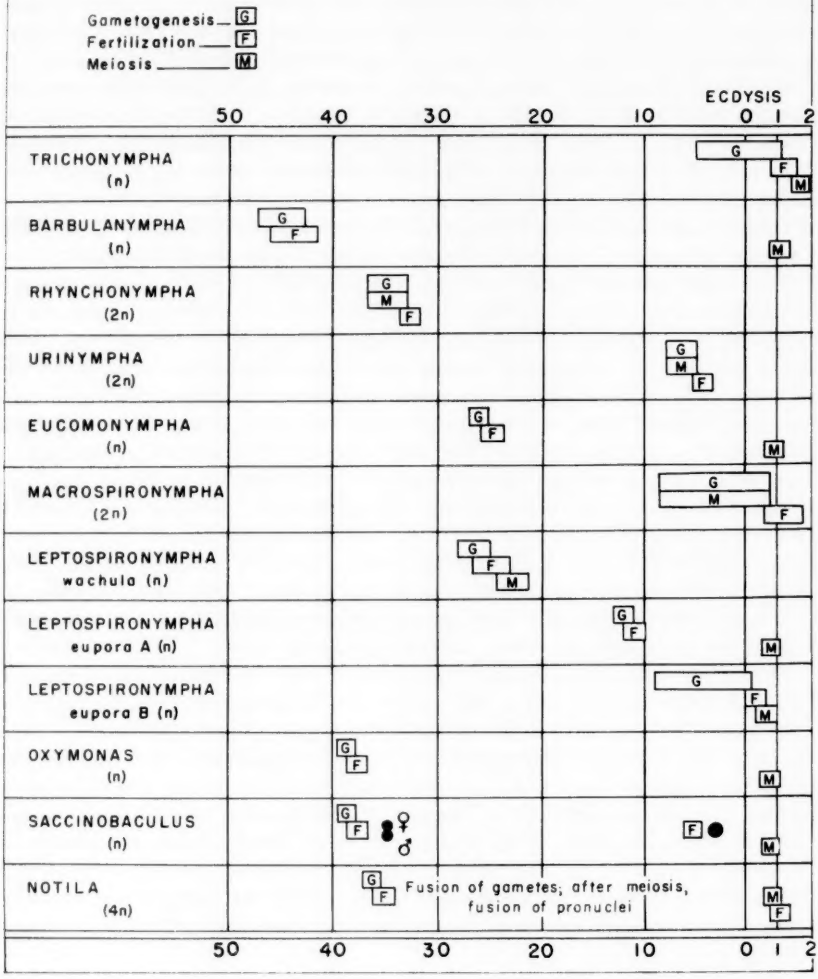
The second series of experiments dealt with intermolt 4th and 5th instar nymphs in which none of the protozoa had begun a sexual cycle. When nymphs of this type were given 100 units of ecdysone, no ecdysis occurred, even in 100 days (most of the ecdysone, as indicated by repeated small doses, probably disappears within 10 days). However, sexual cycles occurred in the protozoa, and all the cycles were completed within 7–8 days after ecdysone was given. This means the protozoa are more sensitive to ecdysone than their host. One hundred units is not enough to carry such nymphs through the molting period to completion (ecdysis), but it is enough to induce sex in their protozoa. The difference in the results of the first and second series of experiments lies in the fact that the nymphs of the first series of experiments were producing ecdysone of their own when this hormone was given them. The amount given, plus what they were producing themselves, was enough to produce ecdysis, and in a much shorter time than would have occurred otherwise. The administered ecdysone served as a booster shot.

The second series of experiments shows that, under experimental conditions, it is not necessary for a roach to undergo ecdysis to induce sex in its protozoa. Beginning about 4 days before ecdysis, the fluid of the hind-gut gradually becomes more viscous. This is true of both natural and experimental ecdyses. But this increase in viscosity did not occur in the intermolt nymphs of the second series of experiments; yet sex occurred just the same. Hence, increase in viscosity plays no role in the induction of sex in the protozoa. It was also shown that the number of organisms present in the hind gut is totally unrelated to the induction of sex. This was done by removing the protozoa from a nymph with oxygen[7] and replacing them with a small number of protozoa from an adult roach (for methods of procedure *vide infra*).

The third series of experiments are the most crucial ones of all. When adult roaches were each given 2,000 units of ecdysone, sex was induced in their protozoa, and with amazing rapidity. Most of the individuals of three genera, *Barbulanympha*, *Saccinobaculus*, and *Oxymonas*, underwent haploid gametogenesis and many examples of fertilization were present within 3 hours after ecdysone was injected. The other genera, as under natural conditions (Table 1) were slower in beginning and completing their sexual cycles. Since adults have no prothoracic glands and hence, unlike nymphs, are incapable of producing any ecdysone of their own, this series

TABLE I

Relation, in days, of the molting period of *Cryptocercus* to sexuality in its protozoa



of experiments shows clearly that ecdysone alone has the ability to induce sex in the protozoa of *Cryptocercus*.

In the fourth series of experiments 100 units of ecdysone were given nymphs from 2–10 days following ecdysis. It should be noted that two days after ecdysis, the protozoa, as shown in Table 1, have completed their sexual cycles. All of the 10 nymphs used in these experiments failed to undergo ecdysis, but, just as in the intermolt nymphs of the second series of experiments, all genera of their protozoa

underwent sexual cycles. Thus, experimental sexual cycles may be induced immediately following the completion of natural ones. And if a large dose of ecdysone is given the induction of sex occurs quickly. For example, two 4th instar nymphs were given 500 units each 5 days after ecdysis. One, which was sacrificed 3 hours later, had many zygotes of *Saccinobaculus* and *Oxymonas;* the other, sacrificed 29 hours later, had *Barbulanympha* that had completed their sexual cycle, and *Trichonympha* that had nearly finished gametogenesis.

In a final experiment 2,000 units of ecdysone were given a last instar nymph 6 hours after ecdysis. Exclusive of three species, the protozoa of such a host still must carry out either meiosis or fertilization before their sexual cycles are completed. The haploids, except for *Leptospironympha wachula*, must undergo meiosis, and the diploids, except for *Urinympha* and *Rhynchonympha*, must undergo fertilization. *Trichonympha* must complete the cytoplasmic changes that differentiate its gametes into male and female, and undergo both fertilization and meiosis. We were not able, however, to determine precisely what happens to these uncompleted sexual processes, owing to the exhaustion of our supply of ecdysone at this point in the experiments. Only one definite observation was made: the nuclei of some genera become greatly enlarged.

Inability to continue experiments with ecdysone caused us to return to experiments which, save for a few details, were completed previously. Since these experiments dealt with the role of the molting hormone in the 3 phases of a sexual cycle, namely, gametogenesis, fertilization, and meiosis, they should be reported here. The two most suitable genera for such experiments are *Barbulanympha* and *Trichonympha*. Both are haploids and, as in all haploids, gametogenesis and meiosis are separate processes. A method had already been developed for transferring the protozoa from one host to another quickly and easily.[6] A thoroughly reliable method was worked out many years ago[7] for killing all the protozoa of the recipient host before the transfer from the donor was made. It thus became a simple matter to take protozoa after gametogenesis was completed in their nymphal host and implant them in the hind-gut of an adult host in which no molting hormone was present. The protozoa were removed from such adults at rather frequent intervals for microscopic examination (phase contrast and fixed and stained preparations) to determine whether the incomplete phases of the sexual cycles were completed or not. The result was that in *Trichonympha* both fertilization and meiosis can be completed in a host without molting hormone, and that in a similar host *Barbulanympha* is able to undergo meiosis. Hence, we were able to conclude that the molting hormone is responsible for only the gametogenesis phase of the sexual cycles of these two genera. The same is very probably true for the other genera in *Cryptocercus*, remembering, of course, that in the diploids gametogenesis and meiosis occur concomitantly. In diploids, then, all phases of the sexual cycle other than fertilization are induced by the molting hormone. And, since the molting hormone is ecdysone, we may conclude that ecdysone produces gametogenesis in haploids and both meiosis and gametogenesis in diploids.

Discussion.—There is general agreement that ecdysone is the growth and differentiation hormone that is responsible for carrying an insect through nymphal or larval development to an adult. The fact that this hormone is also capable of producing growth and sexual differentiation in the protozoan flagellates that live

in the roach *Cryptocercus* is of great interest. It is not just an occasional flagellate in the jam-packed insect gut that responds to this hormone; every individual flagellate present, except an occasional one of the genus *Trichonympha,* responds. But why do these protozoa respond to ecdysone by changing their method of reproduction from asexual to sexual? In the absence of ecdysone all reproduction is asexual, that is mitotic, and is asynchronized. Each parent cell produces daughters like itself. And not more than 1 to 2 per cent of the organisms present are ever in any stage of reproduction at the same time. On the other hand, the response to ecdysone is sexual reproduction which is closely synchronized in all species of each genus. A parent cell, under the influence of ecdysone, not only produces daughters that are greatly different from itself but also daughters much unlike each other, one being a male gamete and the other a female. These gametes, in both their morphology and their physiology, plainly differ from each other at three levels, cytoplasmic, nuclear, and chromosomal. In some genera, the gametes differ from the parent cell that produces them so much that if one did not observe this change one would place the parent cell in one genus and its gametic daughters in another.

At present, one cannot explain how ecdysone acts as such a potent cellular differentiating agent on insect cells and on protozoan flagellates. Protein binding may well be an essential feature of ecdysone activity. Further study should give information on its mode of action. The protozoa will probably provide better material for such a study than insect cells because their cells are immense in comparison to those of insects, and the tremendous differentiations which they undergo all occur in a single generation.

There will doubtless be some who will ask whether the action of ecdysone on these protozoa is direct or indirect. Our present knowledge is too meager, it seems to me, to warrant a discussion of this question. There are probably several profound chemical processes concerned in gametogenesis—in protozoa as well as higher organisms.

Is ecdysone responsible for the polyploidy so common in certain tissues of many insects? For example, the tissues of the gut and Malpighian tubules consist of a single layer of cells which do not divide from the first to the last instar. However, they do enlarge with each instar, at which time the polyploidy of their nuclei also increases. This increase, both in ploidy and cell size, occurs shortly before ecdysis, and thus coincides with a high titer of ecdysone. This behavior of cytoplasm and chromosomes may be produced in response to ecdysone. The cells of the fat body behave in a similar manner, and in addition there is differentiation in them.

The giant polytene chromosomes of the salivary glands may also be produced in response to ecdysone. Their size increases at the ecdysis of each instar, at which time they are subjected to a high titer of ecdysone.

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*MULTIPLE FORMS OF ENZYMES: TISSUE, ONTOGENETIC, AND SPECIES SPECIFIC PATTERNS**

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The fundamental metabolic activities of organisms are very similar and consequently enzymes catalyzing identical reactions may be found in many different organisms and in many different tissues within an organism. When subjected to a variety of physical,[1-4] chemical,[5, 6, 7] or serological[8-17] tests enzymes from different organisms are commonly found to be different from each other even though catalyzing the same chemical reaction. In view of the demonstrated genetic control of protein synthesis[18] it is not surprising that differences should exist in the structure of homologous enzymes or proteins synthesized by animals of different species[19-24] or even by animals of different genotype within the same species.[25-29] Rather surprising, however, is the evidence demonstrating that several enzymes exist in multiple molecular forms not only within a single organism but even within a single tissue. Among the enzymes that have been reported to exist in separate molecular types within a single tissue are esterase,[30, 31] ribonuclease,[32, 33, 34] pepsin,[35] chymotrypsin,[36] trypsin,[37] lysozyme,[38, 39] cytochrome C,[40] xanthine dehydrogenase,[41] malate dehydrogenase,[42, 43, 44] and lactate dehydrogenase.[2-4, 42-49] Likewise, in yeast distinct molecular types of phosphoglyceraldehyde dehydrogenase[50] and of enolase[51] have been identified. The existence of each of these enzymes as a family of closely related but distinguishable molecular types suggests the need for an extension of the classification of enzymes beyond that based on substrate specificity alone. We propose, therefore, to use the term *isozyme* to describe the different molecular forms in which proteins may exist with the same enzymatic specificity.

In the present investigation three dehydrogenase enzymes—lactate dehydrogenase (LDH),† malate dehydrogenase (MDH), and isocitrate dehydrogenase (IDH)—have been resolved into physically distinct forms, that is, into isozymes. In addition to the dehydrogenases, three enzymes with broad substrate specifici-

ties—that is, alkaline phosphatase, peroxidase, and esterase—have also been resolved into multiple molecular forms. However, each of these enzymes with broad substrate specificity is probably a family of enzymes the members of which have overlapping but distinct patterns of substrate specificity, and therefore they should not at present be classified into isozymes. The esterases, like the LDH isozymes, do indeed exhibit tissue specific patterns that arise gradually during the course of embryonic development, but the dehydrogenases owing to greater substrate specificity are more suitable material for studying the origin and tissue distribution of closely related but distinct molecular species. In this report the LDH isozymes have been studied principally with special reference to their tissue and species specificity and to their ontogeny.

Partly because of its use as a diagnostic tool in human disease LDH has attracted considerable attention and numerous investigators have demonstrated the occurrence of this enzyme in multiple forms. The work of Wieland and Pfleiderer[2] and of Pfleiderer and Jeckel[3] has been especially noteworthy in demonstrating the species and tissue specificity of the isozymes of LDH. We have been able to confirm their general results. However, the greater resolving power and precision of a combination of electrophoretic and histochemical techniques used in the present investigation have enabled us to amplify and clarify their results. For example, these investigators found one LDH isozyme in pig heart and two in beef heart, whereas we find three and four, respectively. Moreover, comparative electrophoretic mobilities can be more clearly ascertained by the starch gel electrophoresis method used in the present investigation.

Materials and Methods.—One of the best methods for resolving complex mixtures of proteins is zone electrophoresis in starch gels. This method developed initially by Smithies[52] for separating serum proteins has proved valuable in the separation of several enzymes[30, 31, 53] from tissue homogenates. The details of procedure have been set forth previously.[31, 52] By these methods of starch gel electrophoresis about 1 mg of protein in a homogenate volume of 0.02 ml may be resolved in six hours. After electrophoretic resolution of the homogenate the starch block is split horizontally and one half stained with amido black to reveal the location of protein bands. The remaining half is then used for locating enzymatic activity. The identification of each kind of enzyme presents problems peculiar to itself. By the use of conventional histochemical methods several enzymes have been identified directly on the starch gel—namely, esterase, phosphatases, tyrosinases, and peroxidases. The starch strip containing the visualized enzymes has been called a *zymogram*.[30]

Methods for identifying dehydrogenases directly on the starch gel all proved unsatisfactory and so an indirect method was developed that should have general applicability to any electrophoretically mobile enzyme using DPN or TPN as cofactor. In this method the starch strip, after electrophoresis, is placed in a tray and overlayed to a depth of 2 mm with an agar solution (1.5 per cent) at 45°C containing the reactants. For LDH these are lactate, DPN, hydrazine, diaphorase, methylene blue, and neotetrazolium chloride. Other enzymes require somewhat different reactants. The tray is then covered quickly to exclude oxygen, cooled in an ice bath until the agar gels, and then placed in a dark incubator at 37°C. Within about one hour the location of dehydrogenase activity in the starch is

revealed by the precipitation of a purple formazan dye in the agar directly above the area of the starch containing a dehydrogenase.

A schematic representation of the reactions involved is shown in Figure 1. This method essentially detects DPNH or TPNH—hence its general applicability. The sensitivity can be very great since the accumulated product of a prolonged enzyme reaction is measured. LDH activity of less than a gamma of protein is readily detected.

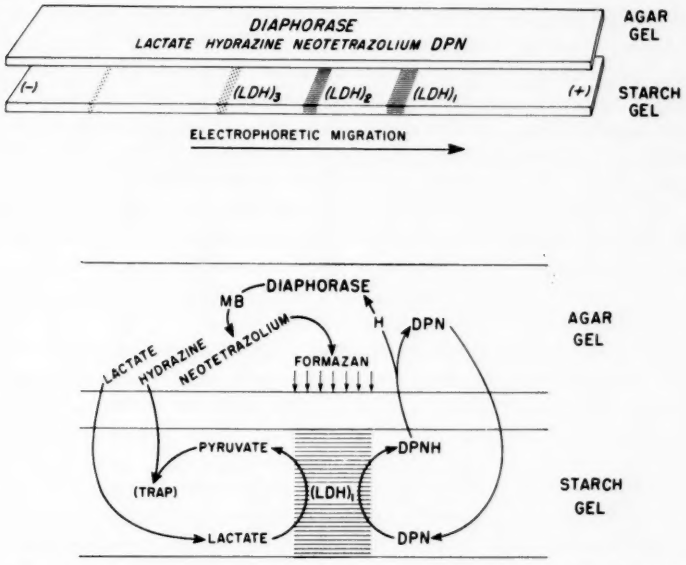

FIG. 1.—Schematic representation of the reactions involved in localizing LDH isozymes after they have been separated by electrophoresis in a starch gel. A 1.5 per cent solution of agar containing the following reactants is poured over the starch and quickly cooled: sodium-DL-lactate, 0.1 *M;* DPN, 0.3 mg/ml; hydrazine (neutralized), 0.1M; diaphorase, 0.6 units/ml.[54]; neotetrazolium chloride, 0.5 mg/ml; phosphate buffer pH 7.4, 0.67 *M;* methylene blue, 0.003 mg/ml. This method detects DPNH or TPNH since these substances reduce neotetrazolium to an insoluble formazan dye.

Once an enzyme has been located on one half of the starch strip the corresponding area of the other half may be cut out, frozen to break up the gel structure, and the enzyme eluted for further tests. An additional, though less satisfactory method in our hands, for locating dehydrogenases is based on the loss of fluorescence under ultraviolet light as DPNH is oxidized to DPN.[2] By this method the dehydrogenases show up as dark bands on a fluorescent background.

Results.—Tissue and species specific patterns of LDH isozymes: Purified, crystalline beef heart LDH was electrophoretically resolved into five distinct protein bands, four of which had enzymatic activity. The band at position III separates into two components after prolonged electrophoresis, but is pictured as occupying a single position in Figure 2. Crude homogenates of beef heart, although containing many different proteins, were resolved into a similar pattern of LDH isozymes.

However, an isozyme from the crude homogenate appeared at position V, and this isozyme apparently was lost during the purification of the crystalline preparation. On the other hand the crystalline preparation contained a minor isozymic component appearing at position IV that was not evident in the crude homogenate. This new component may be a preparative artifact or may result from the concentration of an isozyme that is too dilute to be detected in the crude homogenate.

The LDH isozyme pattern of beef heart was compared with the pattern of isozymes from the heart of sheep, pig, mouse, and rabbit (Fig. 2). Each pattern is unique, though the isozyme at position II is present in each species. Thus far, seven electrophoretically distinct mammalian LDH isozymes have been recognized. Two of these from beef heart are closely adjacent in position III but the remaining five occupy distinctly separated electrophoretic positions. Some tissues contain only one, others as many as five of these isozymes. The apparent relative amount of the different isozymes present also varies considerably between different tissues. An examination of the LDH isozyme pattern of seven different tissues of the pig shows each of these patterns to be tissue specific (Fig. 2). Heart, skeletal muscle, smooth muscle of the pyloric sphincter, diaphragm, liver, kidney, and brain have all been analyzed for their LDH isozymes and each tissue is to some degree different from all the others. It is obvious that tissues differ both in the particular isozymes they contain and in the relative proportions of these isozymes.

LDH isozyme patterns in embryonic tissues: Since mature tissues differ from each other in isozymic content these differences must reflect divergent ontogenies. Accordingly embryonic tissues should contain isozymic patterns differing from adult tissues. This expectation was realized in an analysis of isozymic patterns in the tissues of embryonic pigs of 12 cm crown-rump length. A comparison of the isozymic pattern of these embryonic tissues with homologous adult tissues is shown in Figure 2. A photographic reproduction of several zymograms of LDH isozymes is also shown in Figure 3. It is apparent that the final adult pattern is reached by both gains and losses in the isozyme repertory of embryonic tissues. Embryonic heart, an active functioning organ at this embryonic stage, contains a larger number of LDH isozymes than adult pig heart. Skeletal muscle, on the other hand, contains fewer isozymes than adult muscle. Particularly, the single isozyme that migrates toward the cathode at pH 8.6 and is characteristic of adult skeletal muscle is not present at this embryonic stage. Other differences between embryonic and adult tissue, particularly in the relative enzymatic activity disposed in each isozymic band, are shown in the zymograms of Figure 2. Characteristic of several embryonic pig tissues is the presence of an isozyme at position III. Curiously, this isozyme later disappears in adult pig tissues although adult tissues of other species retain an isozyme at this position.

The substrate specificity of the LDH isozymes of beef heart was tested qualitatively with eight substrates: lactate, β-phenyllactate, α-hydroxybutyrate, α-hydroxy-N-caproate, α-hydroxyvalerate, α-hydroxyisovalerate, α-hydroxyisobutyrate, and α-hydroxy-β-methylvalerate. All these substrates were oxidized in the presence of the LDH isozymes though not at the same rate. However, all of the LDH isozymes were alike in the relative catalytic efficiency displayed toward these different substrates. Thus no difference in pattern of substrate specificity was apparent.

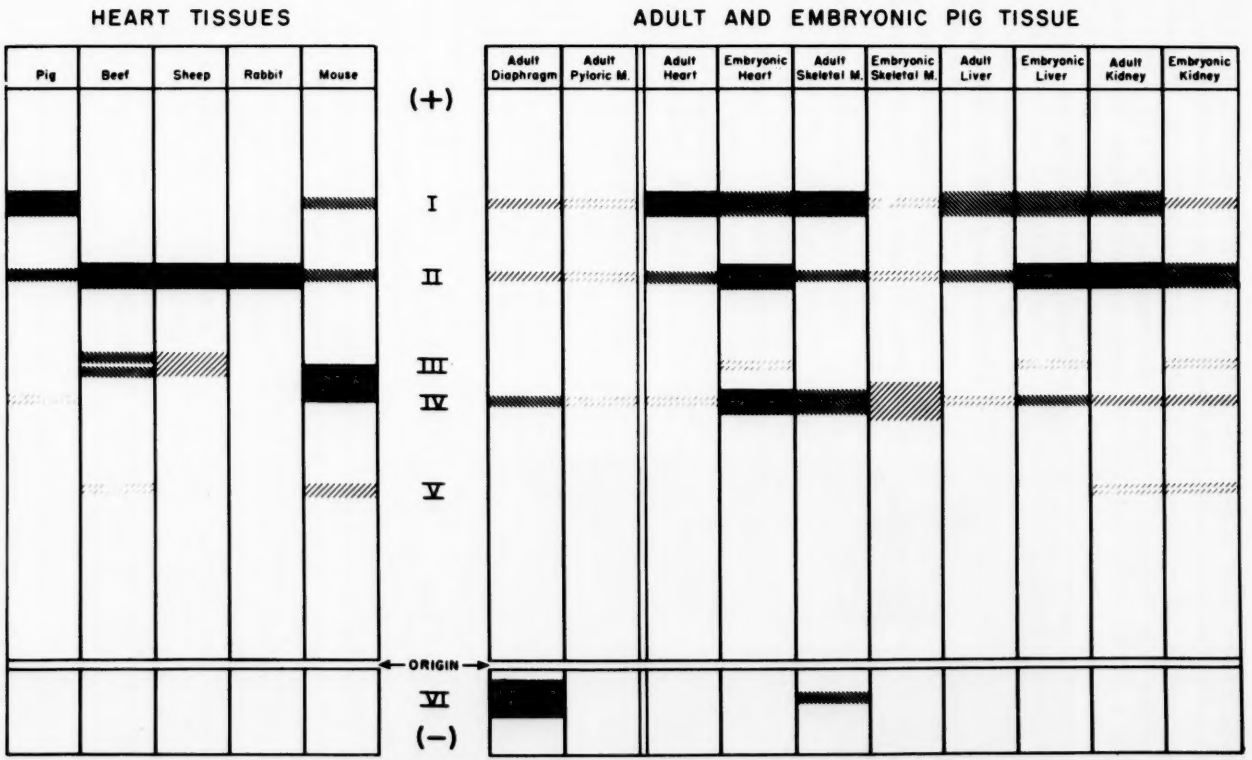

FIG. 2.—Diagrammatic representation of the intensity, size, and location of LDH isozymes from several tissues as seen in zymograms made by the procedure outlined in Figure 1. Electrophoretic resolution was achieved in six hours at a voltage drop of 6 v/cm and at a pH of 8.6. Under these conditions all but one of the isozymes migrated toward the anode.

Multiple forms of other enzymes: In addition to LDH several other dehydrogenase enzymes have been investigated by the same techniques to determine whether they too exist in isozymic forms. Isocitrate dehydrogenase from pig heart was resolved into three isozymes and malate dehydrogenase into at least two. In assaying for IDH, TPN and a TPN diaphorase from spinach leaves were employed.[55] These different dehydrogenase isozymes (whether of LDH, MDH, or IDH) all proved to be electrophoretically distinct. Glutamate dehydrogenase appeared to be electrophoretically immobile in the starch gel since all activity was confined to the area in which the sample was inserted.

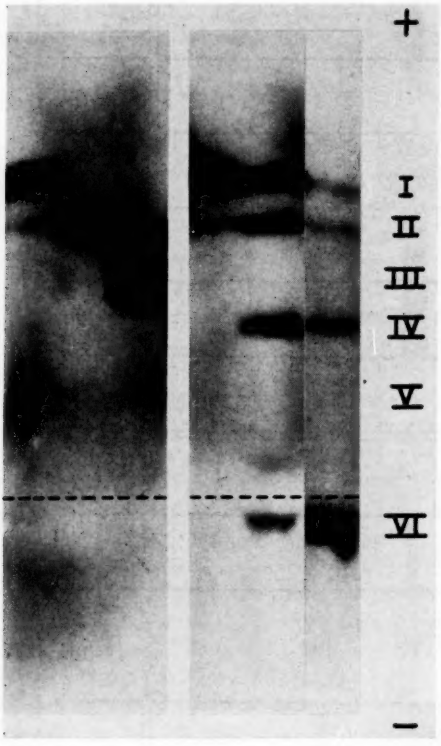

FIG. 3.—Photographic reproduction of zymograms of LDH isozymes. On the left is a compound zymogram of pig, rabbit, and beef heart in that order showing the comparative positions of the constituent isozymes. On the right are three zymograms showing LDH isozymes in adult heart and skeletal muscle and in the diaphragm of the pig. The faintest band in the pig and beef heart zymograms is not apparent in these photographs though evident in the original preparations. In obtaining compound zymograms homogenate samples are adsorbed on filter paper and inserted adjacent to one another in a transverse slit in the starch block.

Multiplicity of molecular forms, of course, is not confined to the dehydrogenases. Enzymes with broad substrate specificities may also be resolved into separate molecular types, but these electrophoretically separated molecular species probably represent distinct enzymes rather than isozymic forms of the same enzyme. Further work on their substrate specificity is needed to clarify their status. Previously Markert and Hunter[31] demonstrated the tissue specific patterns of esterases in the mouse and revealed the existence of more than ten electrophoretically distinct esterases. In the present investigation alkaline phosphatase from tissue homogenates of mice was resolved into three phosphatases that occurred in tissue specific patterns. These enzymes were visualized on the starch gel by the histochemical techniques used for esterases except that α-naphthyl-phosphate was used as substrate. A commercial preparation of horseradish peroxidase was resolved into ten bands all showing peroxidase activity as judged by the benzidine test. At a pH of 8.6 seven of these peroxidases migrated toward the cathode and three moved toward the anode.

Discussion.—Gene-enzyme relationships: The genetic control of the synthesis of

specific proteins has been repeatedly demonstrated and in a few cases changes in the specific properties of a protein have been correlated with corresponding changes in a gene. For example, several varieties of adult and fetal hemoglobin have been identified and shown to be under genetic control with heterozygous individuals producing the two alternative types of hemoglobin.[18] The existence of isozymes raises the question of whether each of these also is controlled by a separate gene or whether they are all modifications of a single gene product. However, it is implausible to consider the multiplicity of isozymes as reflecting a heterozygous genetic constitution since the many animals examined thus far could scarcely have been so uniformly heterozygous. Indeed the mice were from highly inbred colonies. Nevertheless, a multiple gene basis for isozymes is suggested by the existence of independent genes for the synthesis of fetal and adult hemoglobins. Especially relevant is the fact that these genes for hemoglobin synthesis function at different periods during ontogeny. Certain isozymes also appear to be characteristic of specific stages of development.

The genetic basis for isozyme synthesis cannot be settled at present and must await the discovery of isozymic differences in organisms that can be subjected to genetic analysis. However, it is worth noting that each species has its own characteristic isozymic pattern, presumably reflecting the genetic makeup of the species. Perhaps significance can be seen in the fact that the LDH isozymes of the mammalian species examined occupy only a limited number of electrophoretic positions. That is, although all of the species possess some isozymes in common they differ in the possession of others and in the relative proportions of those present. These overlapping but distinct patterns of isozymes in genetically different animals are amenable to either of two genetic interpretations: each isozyme may be attributed to a single gene, with species differences due to the particular genes possessed, or alternate forms of a gene may produce somewhat different constellations of isozymes, each species then having a uniquely characteristic gene for synthesizing its own array of LDH isozymes.

Isozymes and cellular differentiation: Regardless of the genetic basis for isozyme synthesis it is clear that the genetic potential is realized through the processes of cellular differentiation. Each tissue has its own characteristic pattern of isozymes and this pattern changes during embryonic development until the adult configuration is reached. Embryonic tissues, depending upon the one selected, may possess either more or fewer isozymes than corresponding adult tissues, and the relative amounts of the isozymes also vary in accord with the stage of development. This tissue specificity of isozyme pattern may be based on populations of different kinds of cells with each single cell producing only a single LDH isozyme or alternatively a single cell may produce several isozymes. No evidence is yet available to distinguish critically between these alternatives but the second one seems more probable in view of the observation that embryonic pig heart contains a larger number of isozymes than the adult heart (the reverse is true of skeletal muscle). Moreover, the relative proportions of the various isozymes in embryonic and adult tissues is conspicuously different. If changing cell populations are to account for changing isozymic patterns then these shifts in cell populations would have to be much greater than direct observations of embryonic development would suggest. Such drastic changes in cell populations seem unlikely. More plausible is the hypothesis that

the isozymic pattern of a tissue reflects the state of differentiation of its cells.

If isozymic multiplicity is viewed as a function of cellular differentiation rather than as a rigid expression of genetically specified multiple enzyme-forming sites, then several hypotheses may be considered. During the course of changing cellular metabolism the same enzyme-forming site may produce a variety of closely related but distinguishable protein molecules depending upon the raw material made available to it, or perhaps the protein-forming mechanism itself is subject to slight structural variations with a consequent variation in its products. After an enzyme molecule has been synthesized it is integrated into the structure of the cell. Various modes of attachment within the cell may account for changes in reactive groups on the molecule, thus changing the net molecular charge and hence the electrophoretic mobility of the molecule. Electrophoretic mobility is a function of molecular size as well as charge and the several isozymes might be interpreted as reflecting merely different aggregates or polymers of a single molecular species. However, the monodisperse behavior in the ultracentrifuge of crystalline preparations, containing several isozymes, seems to exclude this possibility.[45]

Differences among isozymes: In addition to distinguishing LDH isozymes on the basis of their electrophoretic properties efforts have also been made to distinguish them by their enzymatic behavior. LDHs from different species, from beef heart and rabbit skeletal muscle, were shown by Kaplan *et al.*,[6] to reduce analogues of DPN at different relative rates. Likewise Pfleiderer, *et al.*,[7] showed that pig heart, rabbit skeletal, and rat skeletal muscle LDH all show different degrees of inhibition with 1-fluoro-2,4-dinitrobenzol. It is now clear that these two investigations were dealing with mixtures of isozymes but even so the differences observed must reflect differences in individual isozymes. In two investigations individual LDH isozymes were separated from the tissues of a single animal and shown to be different. Wieland and Pfleiderer[2] found a correlation between degree of sulfite inhibition and electrophoretic mobility. Vesell and Bearn[43, 44] resolved by starch paste electrophoresis three LDH fractions from human blood. These fractions were eluted and shown to each have a distinct pH optimum. However they responded alike to two inhibitors, EDTA and PCMB.

The fact that difference in enzymatic behavior, even though slight, can be shown to distinguish isozymes raises the question of the sharpness of the boundary separating a family of isozymes from distinctly different enzymes. It seems unlikely that further research will reveal a continuum of enzymatic properties ranging from one enzyme to the next. However, there may be considerable variation in the degree of uniformity characterizing different families of isozymes. Some may be essentially identical enzymatically, others may diverge so much as to more properly be described as distinct enzymes (esterases for example). Nevertheless, the concept of enzymes existing as families of isozymes seems useful and significant today especially in view of the tissue-specific patterns of these isozymes.

Serological specificity of isozymes: In an attempt to characterize homologous enzymes from different sources investigators have frequently resorted to serological methods. Generally, antisera prepared against an enzyme from one species will not cross-react with homologous enzymes from distantly related species.[9, 10] However, some cross-reaction is commonly observed when the species are closely related (see Cinader[8] for general discussion). For example, Gregory and Wróblewski,[11]

using antisera obtained from chickens injected with rabbit muscle LDH, demonstrated varying degrees of cross-reaction between these antisera and LDHs obtained from five different mammalian species. A similar cross-reactivity was shown by Henion and Sutherland[12] for phosphorylases extracted from the tissues of the dog, rabbit, and cat. These investigators also demonstrated that the phosphorylases from different organs of the same animal are immunologically different though cross-reactive in varying degrees. Serological distinctions have also been shown among alkaline phosphatases extracted from different human tissues (and also from tissues of the dog).[56] Wide variation was noted in the degree of cross-reaction between heterologous antisera and the different tissue phosphatases. The results of these tests suggested that human serum phosphatase was a mixture of types found singly in other tissues.

These results with the LDH, phosphorylase, and phosphatase antisera can perhaps be interpreted in the light of isozymic patterns as merely reflecting the degree to which the different tissues share a common repertory of isozymes. Failure to obtain complete cross-reaction may merely indicate the existence of additional isozymes in the tested tissue that were not present in the immunizing extract. This seems particularly probable as an explanation of partial cross-reactions obtained with enzymes from different tissues of the same species. Whether electrophoretically homologous isozymes from different species are serologically identical has not yet been demonstrated, but work on this problem is now proceeding in this laboratory.

The generality and clarity of the picture presented by the existence of patterns of isozymes that are tissue and species specific and that also change during embryonic development poses significant problems for our understanding of the genetic and embryological regulation of enzyme synthesis. We have not as yet gained any insight into the mechanism by which isozymes arise but any concept of cellular differentiation will have to account for them.

Summary.—(*1*) A search of the literature reveals that many different enzymes have each been resolved by physico-chemical techniques into several distinguishable molecular types. We propose to call these molecular types, *isozymes*.

(*2*) From tissue homogenates three dehydrogenases (LDH, MDH, IDH) were resolved into their component isozymes by zone electrophoresis in starch gels. The dehydrogenase isozymes were then localized on the starch by a new method that should be applicable to any electrophoretically mobile enzyme using DPN or TPN as a cofactor.

(*3*) Lactate dehydrogenase obtained from the tissues of several different species was electrophoretically separated into several molecular types (isozymes) each with the same substrate specificities. The pattern of LDH isozymes is species and tissue specific and changes during the embryological differentiation of the tissue.

(*4*) Esterase, peroxidase, and phosphatase were also resolved into separate molecular types by electrophoresis but each of these enzymes is probably a family of enzymes the members of which have characteristic but overlapping substrate specificities.

(*5*) Genetic, embryological, physiological, and serological considerations are presented in an effort to account for specific patterns of isozymes.

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† The following abbreviations have been used: LDH, lactate dehydrogenase; MDH, malate dehydrogenase; IDH, isocitrate dehydrogenase; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide.

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INTERACTION BETWEEN FORMALDEHYDE AND TOBACCO MOSAIC VIRUS

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In preparation for a study of the uptake of formaldehyde by polio virus, the interaction between formaldehyde and tobacco mosaic virus was investigated. This reaction has been variously observed, e.g., in terms of biological inactivation,[1–4] alterations in electrophoretic pattern,[2, 3] ultraviolet absorption,[5] and reactions[1, 3] of protein or amino groups, but no information is available as to the extent of the intermolecular combination. This gap in our knowledge is due to the small amount of formaldehyde reacting and the analytical difficulty in measuring this small quantity in the presence of a huge excess of formaldehyde.

Materials and Methods.—This problem has been approached with the aid of carbon-14 labeled formaldehyde (specific activity $\simeq$ 3.5 mc/mmole), from which stock solutions of formaldehyde varying in total concentration (C_0) from 50 μg HCHO/ml to $\simeq$ 40,000 μg HCHO/ml were prepared by addition of unlabeled reagent grade formaldehyde. One-ml portions of these solutions were added to 1 ml solution of a purified masked strain of TMV[6] in 0.2 *M* pH 7.0 phosphate buffer containing 2 mg virus/ml in 15-ml Pyrex centrifuge tubes (stoppered by polythene stoppers). After brief agitations, the tubes were immersed in a thermostated water bath for incubation. Order and rate of addition of components proved to be immaterial. Excess formaldehyde was removed by coagulation of virus by 5 ml of 30 per cent $(NH_4)_2SO_4$, centrifugation, and careful decantation of supernatant fluid. Liquid adhering to the walls of the tube was removed by careful wiping with filter paper. The virus pellet was resuspended in salt solution, and the washing cycle repeated five times. This washing procedure required 5–6 hours and was effective in removing excess formaldehyde. Washed virus was suspended in 3 ml of H_2O, and virus content determined by its ultraviolet absorbance.

Aliquots (1 ml) of virus solutions were placed in $1^1/_8$-in. stainless steel planchets to which were added 1 ml. of a dimedon[7, 8] reagent (1 mg/ml) in very dilute pH 4 acetate buffer (1 mg/ml). After 24 hrs of standing, the water was removed by absorption over P_2O_5. The residues were redistributed with methanol which was

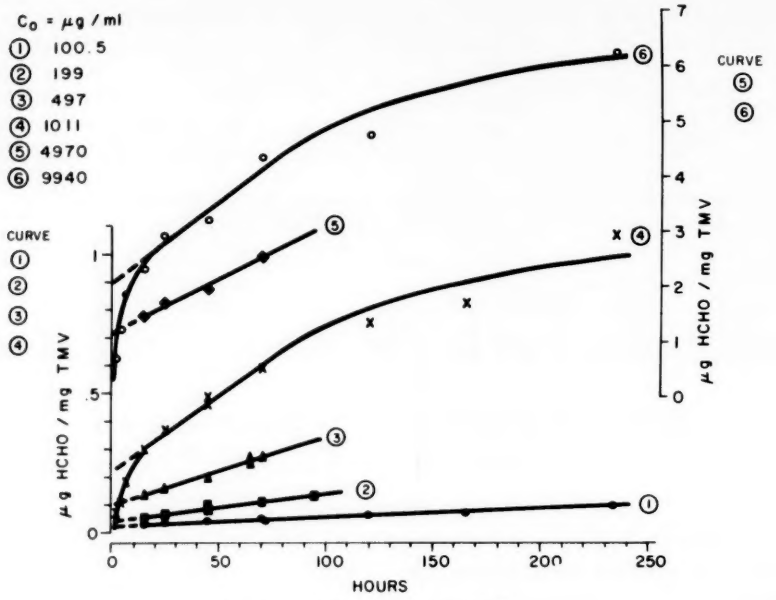

FIG. 1.—HCHO retention by TMV

subsequently removed by evaporation. Radioactivity of planchets was measured in a windowless flow proportional counter. Comparison with appropriate HCHO-C^{14} standards similarly prepared yielded values for HCHO retained by the virus, expressed as μg HCHO/1 mg TMV. Residue weights were seldom in excess of 4 mg, and tests proved self-absorption corrections to be unimportant.

Results.—Results at 37.7°C are tabulated in Table 1. Representative curves are plotted in Figure 1. It is clear that, at every formaldehyde concentration (C_0) studied, initial rapid reaction is superseded in about 15 hrs by a slower reaction characterized by a linear relationship between reaction and time, which in turn gives way to a still slower process after 3–4 days. Except for the two highest HCHO concentrations employed, the slopes of the linear portion are proportional to C_0, as are also the intercepts on the ordinate axis.

Table 2 lists the slopes (F) and intercepts (I) of the linear portions (15 hrs to $\simeq$ 4 days) of the "reaction versus time" curves for all initial formaldehyde concentrations investigated. These values of F and I were obtained by the method of least squares.[9] The intercepts of these curves remain essentially proportional to initial formaldehyde concentration for all C_0 values from 25 μg/ml to 19,900 μg/ml, as witness the relative constancy of the I/C_0 ratios. The slopes vary similarly as is seen from the fair constancy of the F/C_0 ratio for all concentrations studied, except the 1 and 2 per cent formaldehyde solutions in which media the reaction proceeds progressively more slowly. Average values of F/C_0 and I/C_0 are respectively

$$4.21 \times 10^{-6} \frac{\mu g \text{ HCHO/mg virus/hr}}{\mu g \text{ HCHO/ml solution}} \text{ and } 2.03 \times 10^{-4} \frac{\mu g \text{ HCHO/mg virus}}{\mu g \text{ HCHO/ml solution}}$$

TABLE 1

FORMALDEHYDE RETENTION BY TOBACCO MOSAIC VIRUS AT VARIOUS FORMALDEHYDE CONCENTRATIONS (C_0) AT 37.7°C.

	μg HCHO Retained per mg Virus								
Time, hrs.	C_0 = 25 μg/ml	49.5 μg/ml	100.5 μg/ml	199 μg/ml	497 μg/ml	1,011 μg/ml	4,970 μg/ml	9,940 μg/ml	19,860 μg/ml
1 1/2	..	..	0.0063	..	..	0.074	..	0.78	1.60
3	..	..	0.0096	..	..	0.106	..	1.24	2.37
6	..	..	0.0143	..	..	0.174	..	1.96	3.25
15 1/4	0.0062	0.0117	0.026*	0.050	0.139	0.31*	1.54	2.32*	4.26*
23	0.0072	0.0135	0.028	0.055	0.153	0.36	1.72	2.94	4.54
47	0.0092	0.0175	..	0.066	0.193	0.48†	1.94	..	..
				0.092					
47 2/3	..	..	0.036	..	..	0.46	..	3.19	4.90
66	..	..	..	..	0.262	..	..	..	..
					0.242				
71	0.0117	0.0222	..	0.100	0.273	..	2.64	4.39‡	6.00‡
72	..	..	0.047‡	..	..	0.64‡	..	..	..
			0.045			0.58			
95	..	..	..	0.123	..	..	..	..	..
119 1/2	..	..	0.054	..	..	0.74	..	4.76	6.70
168	..	..	0.061	..	..	0.79	..	4.72	7.09
233	..	..	0.085	..	..	1.06	..	6.40	9.28
499	..	..	0.106	..	..	1.31	..	7.45	10.7
864	..	..	0.125	..	..	..	..	8.40	..

* 15 1/2 hrs. † 46 hrs. ‡ 71 2/3 hrs.

TABLE 2

DEPENDENCE OF INTERCEPTS (I) AND SLOPES (F) ON INITIAL FORMALDEHYDE CONCENTRATION (C_0) AT 37.7°C

C_0, μg/ml	I, μg/mg	F, μg/mg/hr × 10^6	I/C_0 × 10^4	F/C_0 × 10^6
25	0.0048	9.6	1.93	3.84
49.5	0.0090	18.5	1.82	3.73
100.5	0.0199	35.9	1.98	3.58
199	0.0351	90.5	1.76	4.55
497	0.0974	236	1.96	4.75
1,011	0.234	532	2.32	5.26
4,970	1.24	1,860	2.48	3.73
9,940	2.04	2,770	2.05	2.79*
19,860	3.92	2,310	1.98	1.16*
Average ± adm.†	..	..	2.03 ± 0.17 μg/mg/virus / μg/ml soln	4.21 ± 0.56 μg/mg virus/hr / μg/ml soln

* Not included in average F/C_0.
† Average deviation from the mean.

Multiplying these figures by 1,000 and by the virus density[10] of 1.37 g/ml converts the units of virus quantity from mg to ml virus. The ratio F/C_0 thus becomes

$$5.77 \times 10^{-3} \frac{\mu g \text{ HCHO/ml virus/hr}}{\mu g \text{ HCHO/ml soluion}} \text{ or } 1.602 \times 10^{-6} \frac{\mu g \text{ HCHO/ml virus/sec,}}{\mu g \text{ HCHO/ml solution}}$$

and I/C_0 assumes the value

$$0.278 \frac{\mu g \text{ HCHO/ml virus}}{\mu g \text{ HCHO/ml solution}}.$$

Discussion.—The dependence of reactivity upon initial formaldehyde concentration suggests the mediation of a diffusion process.[11] In view of the cylindrical shape of the virus, this supposition was investigated by application of diffusion mathematics to the case of an infinite cylinder in which simultaneous radial diffusion (diffusion constant D cm^2 sec^{-1}) and irreversible first order reaction (constant k

sec^{-1}) with respect to formaldehyde occur. For a solid cylinder, the differential equation to be solved is

$$\frac{\delta C}{\delta t} = D\left[\frac{\delta^2 C}{\delta r^2} + \frac{1}{r}\frac{\delta C}{\delta r}\right] - kC, \tag{1}$$

where C is the concentration of formaldehyde within the virus at time t. This case has been solved by Crank[12] who applied the method of Danckwerts[13] to a number of geometric forms. Alternatively, the application of the finite Hankel transform[14] leads to the same result for the cylinder. Neglect of diffusion through the ends of the cylinder introduces an error of only $\simeq 3$ per cent; and for the sake of simplicity, the hexagonal habit and probable helically grooved surface[15, 16, 17] of the virus are ignored. The result of such a calculation is the following expression for total uptake of formaldehyde (M_t) per cm length of virus versus time,

$$M_t = 4\pi DC_0 \sum\left[\frac{kt(k + D\alpha_n^2) + D\alpha_n^2 - D\alpha_n^2 e^{-(k + D\alpha_n^2)t}}{(k + D\alpha_n^2)^2}\right] \tag{2}$$

in which α_n's are obtained from the roots of the Bessel function $J_0(r\alpha_n) = 0$ and r is the radius of the cylinder. The dependence of total uptake upon C_0 is immediately apparent; and both an initial rapid phase in which diffusion dominates, and subsequent slower reaction are predicted.

If M_t is expressed as μg HCHO/ml virus, this expression reduces to equation (3)

$$\frac{M_t}{C_0} = K\left[\sum\frac{Dk}{k + D\alpha_n^2}t + \sum\frac{D\alpha_n^2}{(k + D\alpha_n^2)^2} - \sum\frac{D\alpha_n^2}{(k + D\alpha_n^2)^2}e^{(-k + D\alpha_n^2)t}\right] \tag{3}$$

in which the value of K (4πx cm/ml virus $= 4/r^2$) depends upon the virus size, and is 6.93×10^{12} for a cylinder of diameter 152 A.[18, 19] For long incubation periods, the general expression reduces to the linear equation

$$\frac{M_t}{C_0} = K\left[\sum\frac{Dk}{k + D\alpha_n^2}t + \sum\frac{D^2\alpha_n^2}{(k + D\alpha_n^2)^2}\right] \tag{4}$$

with a reduced slope

$$F/C_0 = K\sum\frac{Dk}{k + D\alpha_n^2}$$

and intercept

$$I/C_0 = K\sum\frac{D^2\alpha_n^2}{(k + D\alpha_n^2)^2}.$$

The observed constancy of F/C_0 and I/C_0 ratios thus follows directly from the mathematical analysis.

An attempt was made to deduce values of k and D from the above expressions and the experimental values of F/C_0 and I/C_0. From the relationship

$$I/C_0 = K\sum\frac{\alpha_n^2}{\left(\frac{k}{D} + \alpha_n^2\right)^2} = 0.278$$

the ratio k/D was obtained by trial and error; and subsequent introduction of this ratio into

$$F/C_0 = K \sum \frac{k}{\frac{k}{D} + \alpha_n^2} = 1.602 \times 10^{-6} \text{ sec}^{-1}$$

yielded a value for the (assumed) first order constant $k = 3.46 \times 10^{-6}$ sec^{-1}, which is independent of the assumed radius (here taken as 76 Å). The value of D calculated in this manner was 1.57×10^{-19} cm^2 sec^{-1}.

Less extensive studies were performed at 25.3° and 6.3°C and at formaldehyde concentrations of 102.5 μg/ml and 1015 μg/ml. Treated as described above, these data yielded values of $k = 1.64 \times 10^{-6}$ sec^{-1} at 25.3°C and 1.39×10^{-6} sec^{-1} at 6.3°C regardless of assumed radius. Calculated diffusion coefficients were 4.22×10^{-20} cm^2 sec^{-1} at 25.3°C and 1.33×10^{-21} cm^2 sec^{-1} at 6.3°C. The activation energy computed from the rates (linear portions) of total formaldehyde (F/C_0) uptake at different temperatures is $\simeq$ 15,000 calories/mole whereas the activation energy computed from the k's at 37.7 and 25.3°C is 11,000 calories/mole. The velocity constant $k = 1.39 \times 10^{-6}$ sec^{-1} obtained at 6.3°C is but little less than the 25.3°C value of 1.64×10^{-6} sec^{-1}. By contrast, the energy of activation of the diffusion process is 20,000–30,000 calories per mole. This high temperature coefficient of diffusion may be indicative of an activated type of diffusion. The low order of magnitude of D is typical of diffusion in a solid medium, which suggests that the virus does not absorb significant amounts of water from the aqueous medium.

Although mathematical analysis is incomplete and other models, such as the hollow cylinder, surface gradients, back reaction, etc. are being investigated, the success of the theoretical expression in predicting the regularities reported and the nature of the dependence of HCHO uptake upon C_0 are evidence for the reality of the diffusion requirement. The existence of the diffusion mechanism is further supported by the course of reversibility experiments in which mixtures of previously incubated TMV and HCHO are diluted and further incubated at the lower HCHO concentration. This diffusion effect is superimposed upon, and perhaps independent of biological inactivation processes. Destruction of infectivity of tobacco mosaic virus proceeds[4] in solutions containing 2 per cent virus and 2 per cent formaldehyde with a first order rate constant of $\simeq 0.8 \times 10^{-3}$ sec^{-1} at 37.3°C (authors' calculations), and has an activation energy between 30° and 40°C of 19,500 calories/mole as compared to 11,000–15,000 calories per mole for the formaldehyde uptake. The more rapid inactivation reaction may involve diffusion (which may be rapid) of formaldehyde through the open ends[17, 20, 21] of the hollow cylindrical TMV molecule to attack the RNA responsible for infectivity.[22] Evidently also only a part of the formaldehyde retained by the virus is involved in detoxification of the virus.

No information concerning the nature of the reactive groups can be derived from these experiments. The maximum number of formaldehde molecules attached to the virus which has been noted to date is 14,000 HCHO per TMV molecule [molecular weight[19] taken as 3.9×10^7] after 21 days of incubation at an initial concentration of $\simeq$20,000 μg HCHO/ml. This figure is by no means limiting since

reaction is still proceeding. In all likelihood, the decrease in rate of uptake subsequent to the first four days represents an independent reaction, or reflects an element of reversibility[23] in the earlier reaction or reactions. That back reaction will cause an apparent diminution in reaction rate can be demonstrated mathematically.

Summary.—The rate of reaction of formaldehyde with a masked strain of tobacco mosaic virus was determined at three temperatures by means of C^{14}-labeled formaldehyde in nine concentrations ranging from 25 μg HCHO/ml to 19.86 mg/ml. At all concentrations, relatively rapid initial reaction was followed in approximately 15 hours by a slower formaldehyde uptake which was observed to be approximately linear with time. After 3 to 4 days the reaction became still slower, but never quite ceased during the 36 days' duration of these experiments.

The slopes of the intermediate linear portions of the reaction curves, as well as their intercepts with the ordinate, were proportional to initial formaldehyde concentration (C_0). This behavior suggests the intervention of a diffusion process. Considering the virus as an infinite cylinder, application of diffusion mathematics to the case of simultaneous irreversible first order reaction with respect to formaldehyde and radial diffusion of formaldehyde into the virus leads to an expression for formaldehyde retention from which values of velocity constant and diffusion coefficient may be deduced.

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CONTENTS

Page

ANTHROPOLOGY.—SYMPOSIUM ON SCIENCE IN SOCIETY 661

SCIENCE IN SOCIETY . . . . . *By Harry L. Shapiro, Chairman* 661

SOME REFLECTIONS ON THE STATE OF SCIENCE IN AMERICA DURING THE NINETEENTH CENTURY . . . . . *By I. Bernard Cohen* 666

SCIENCE IN THE FRENCH REVOLUTION . . . . . *By Charles C. Gillispie* 677

SOCIETY, SCIENCE, AND EDUCATION IN THE SOVIET UNION . . *By Chauncy D. Harris* 684

BACTERIOLOGY.—METABOLIC ACTIVITIES OF SUBCELLULAR PARTICLES FROM *Azotobacter Vinelandii* . . . . . *By Alvin G. Jose, Jr. and P. W. Wilson* 692

BIOCHEMISTRY.—DIHYDROTHIOCTYL DEHYDROGENASE—A FLAVOPROTEIN . . . . . *By Robert L. Searls and D. R. Sanadi* 697

BIOCHEMISTRY.—THE ATP-DEPENDENT REDUCTION OF SULFATE WITH HYDROGEN IN EXTRACTS OF *Desulfovibrio Desulfuricans* . . . . . *By Harry D. Peck, Jr.* 701

GENETICS.—MUTATION INDUCTION AND MACROMOLECULAR SYNTHESIS IN BACTERIA . . . . . *By C. O. Doudney and F. L. Haas* 709

GENETICS.—TRANSDUCTION BY STAPHYLOCOCCAL BACTERIOPHAGE . . . *By M. L. Morse* 722

GENETICS.—ABERRANT TETRADS IN *Sordaria Fimicola* . . . . . *By Lindsay S. Olive* 727

MATHEMATICS.—UNIQUE FACTORIZATION IN REGULAR LOCAL RINGS . . . . . *By Maurice Auslander and D. A. Buchsbaum* 733

MATHEMATICS.—ON THE FALSITY OF EULER'S CONJECTURE ABOUT THE NON-EXISTENCE OF TWO ORTHOGONAL LATIN SQUARES OF ORDER $4t + 2$ . . . . . *By R. C. Bose and S. S. Shrikhande* 734

MATHEMATICS.—WHICH LIE GROUPS ARE HOMOTOPY-ABELIAN? . . . . . *By Ioan James and Emery Thomas* 737

MATHEMATICS.—A QUANTITATIVE FORMULATION OF SYLVESTER'S LAW OF INERTIA . . . . . *By A. M. Ostrowski* 740

PHYSICS.—ENERGY ABSORPTION BY CHARGE CARRIERS OF NEGATIVE EFFECTIVE MASS IN CRYSTALS . . . . . *By C. Kittel* 744

ZOÖLOGY.—SEX INDUCED WITH ECDYSONE . . . . . *By L. R. Cleveland* 747

BIOCHEMISTRY.—MULTIPLE FORMS OF ENZYMES: TISSUE, ONTOGENETIC, AND SPECIES SPECIFIC PATTERNS . . . . . *By Clement L. Markert and Freddy Møller* 753

BIOCHEMISTRY.—INTERACTION BETWEEN FORMALDEHYDE AND TOBACCO MOSAIC VIRUS . . . . . *By Henry T. Meriwether, Jr. and Charles Rosenblum* 763